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PATENT APPLICATION COVER SHEET

DIARYL PEPTIDES AS NS3-SERINE PROTEASE INHIBITORS OF HEPATITIS C VIRUS

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DIARYL PEPTIDES AS NS3-SERINE PROTEASE INHIBITORS OF HEPATITIS C VIRUS

FIELD OF INVENTION

The present invention relates to novel hepatitis C virus ("HCV") protease inhibitors, pharmaceutical compositions containing one or more such inhibitors, methods of preparing such inhibitors and methods of using such inhibitors to treat hepatitis C and related disorders. This invention specifically discloses diaryl peptide compounds as inhibitors of the HCV NS3/NS4a serine protease.

BACKGROUND OF THE INVENTION

Hepatitis C virus (HCV) is a (+)-sense single-stranded RNA virus that has been implicated as the major causative agent in non-A, non-B hepatitis (NANBH), particularly in blood-associated NANBH (BB-NANBH)(see, International Patent Application Publication No. WO 89/04669 and European Patent Application Publication No. EP 381 216). NANBH is to be distinguished from other types of viral-induced liver disease, such as hepatitis A virus (HAV), hepatitis B virus (HBV), delta hepatitis virus (HDV), cytomegalovirus (CMV) and Epstein-Barr virus (EBV), as well as from other forms of liver disease such as alcoholism and primary biliar cirrhosis.

Recently, an HCV protease necessary for polypeptide processing and viral replication has been identified, cloned and expressed; (see, e.g., U.S. Patent No. 5,712,145). This approximately 3000 amino acid polyprotein contains, from the amino terminus to the carboxy terminus, a nucleocapsid protein (C), envelope proteins (E1 and E2) and several non-structural proteins (NS1, 2, 3, 4a, 5a and 5b). NS3 is an approximately 68 kda protein, encoded by approximately 1893 nucleotides of the HCV genome, and has two distinct domains: (a) a serine protease domain consisting of approximately 200 of the N-terminal amino acids; and (b) an RNA-dependent ATPase domain at the C-terminus of the protein. The

NS3 protease is considered a member of the chymotrypsin family because of similarities in protein sequence, overall three-dimensional structure and mechanism of catalysis. Other chymotrypsin-like enzymes are elastase, factor Xa, thrombin, trypsin, plasmin, urokinase, tPA and PSA. The HCV NS3 serine protease is responsible for proteolysis of the polypeptide (polyprotein) at the NS3/NS4a, NS4a/NS4b, NS4b/NS5a and NS5a/NS5b junctions and is thus responsible for generating four viral proteins during viral replication. This has made the HCV NS3 serine protease an attractive target for antiviral chemotherapy.

It has been determined that the NS4a protein, an approximately 6 kda polypeptide, is a co-factor for the serine protease activity of NS3. Autocleavage of the NS3/NS4a junction by the NS3/NS4a serine protease occurs intramolecularly (i.e., *cis*) while the other cleavage sites are processed intermolecularly (i.e., *trans*).

Analysis of the natural cleavage sites for HCV protease revealed the presence of cysteine at P1 and serine at P1' and that these residues are strictly conserved in the NS4a/NS4b, NS4b/NS5a and NS5a/NS5b junctions. The NS3/NS4a junction contains a threonine at P1 and a serine at P1'. The Cys→Thr substitution at NS3/NS4a is postulated to account for the requirement of *cis* rather than *trans* processing at this junction. See, e.g., Pizzi et al. (1994) Proc. Natl. Acad. Sci. (USA) 91:888-892, Failla et al. (1996) Folding & Design 1:35-42. The NS3/NS4a cleavage site is also more tolerant of mutagenesis than the other sites. See, e.g., Kollykhalov et al. (1994) J. Virol. 68:7525-7533. It has also been found that acidic residues in the region upstream of the cleavage site are required for efficient cleavage. See, e.g., Komoda et al. (1994) J. Virol. 68:7351-7357.

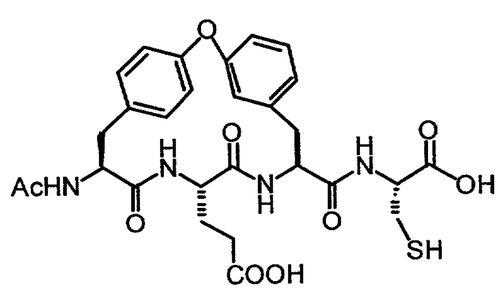
Inhibitors of HCV protease that have been reported include antioxidants (see, International Patent Application Publication No. WO 98/14181), certain peptides and peptide analogs (see, International Patent Application Publication No. WO 98/17679, Landro et al. (1997) Biochem. 36:9340-9348, Ingallinella et al. (1998) Biochem. 37:8906-8914, Llinàs-Brunet et al. (1998) Bioorg. Med. Chem. Lett. 8:1713-1718), inhibitors based on the 70-amino acid polypeptide eglin c (Martin et al. (1998) Biochem. 37:11459-11468, inhibitors affinity selected from human pancreatic secretory trypsin inhibitor (hPSTI-C3) and minibody repertoires (MBip) (Dimasi et al. (1997) J. Virol. 71:7461-7469), cV_HE2 (a "camelized"

variable domain antibody fragment) (Martin et al.(1997) Protein Eng. 10:607-614), and α1-antichymotrypsin (ACT) (Elzouki et al.) (1997) J. Hepat. 27:42-28). A ribozyme designed to selectively destroy hepatitis C virus RNA has recently been disclosed (see, BioWorld Today 9(217): 4 (November 10, 1998)).

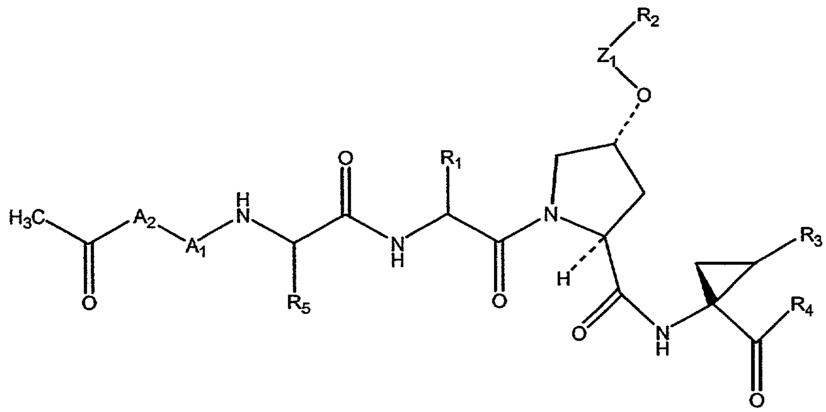
Reference is also made to the PCT Publications, No. WO 98/17679, published April 30, 1998 (Vertex Pharmaceuticals Incorporated); WO 98/22496, published May 28, 1998 (F. Hoffmann-La Roche AG); and WO 99/07734, published February 18, 1999 (Boehringer Ingelheim Canada Ltd.).

HCV has been implicated in cirrhosis of the liver and in induction of hepatocellular carcinoma. The prognosis for patients suffering from HCV infection is currently poor. HCV infection is more difficult to treat than other forms of hepatitis due to the lack of immunity or remission associated with HCV infection. Current data indicates a less than 50% survival rate at four years post cirrhosis diagnosis. Patients diagnosed with localized resectable hepatocellular carcinoma have a five-year survival rate of 10-30%, whereas those with localized unresectable hepatocellular carcinoma have a five-year survival rate of less than 1%.

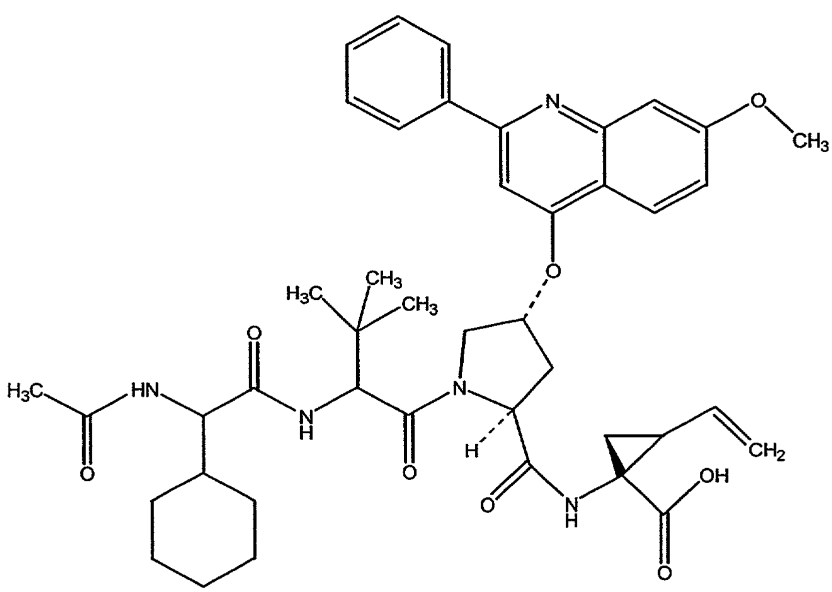
Reference is made to A. Marchetti *et al*, *Synlett*, S1, 1000-1002 (1999) describing the synthesis of bicylic analogs of an inhibitor of HCV NS3 protease. A compound disclosed therein has the formula:



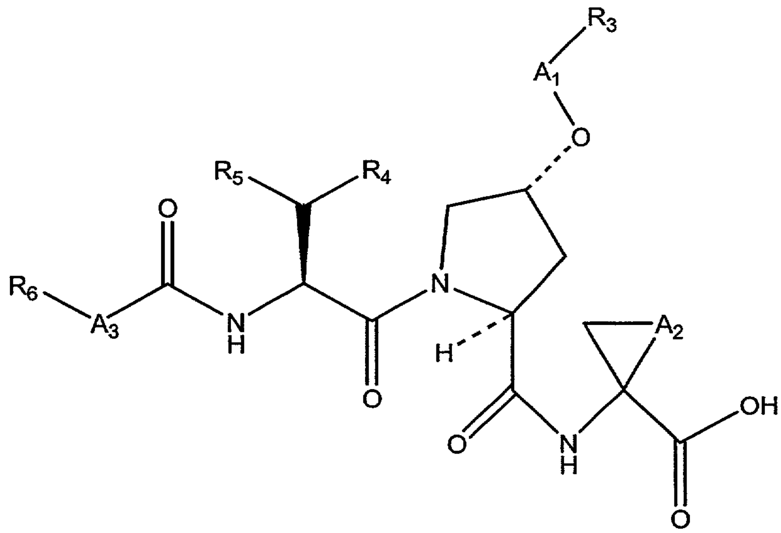
Reference is also made to WO 00/09558 (Assignee: Boehringer Ingelheim Limited; Published February 24, 2000) which discloses peptide derivatives of the formula:



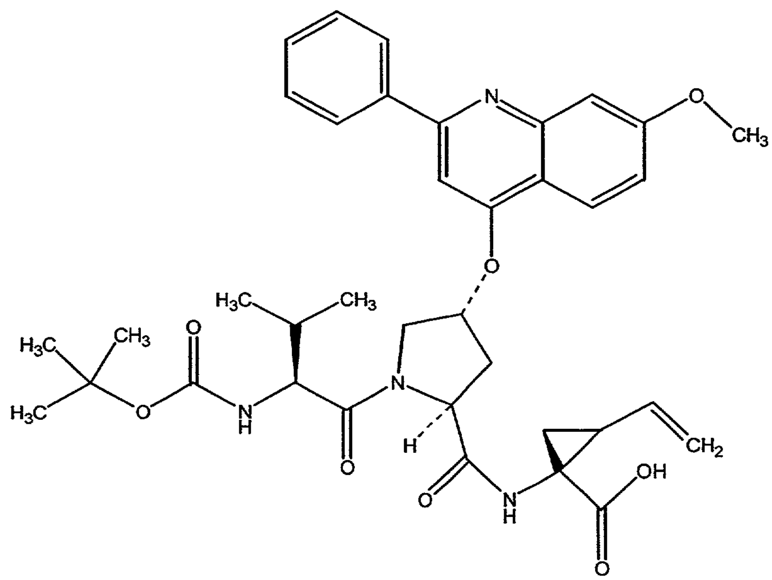
where the various elements are defined therein. An illustrative compound of that series is:



Reference is also made to WO 00/09543 (Assignee: Boehringer Ingelheim Limited; Published February 24, 2000) which discloses peptide derivatives of the formula:



where the various elements are defined therein. An illustrative compound of that series is:



Current therapies for hepatitis C include interferon-α (INF_{α}) and combination therapy with ribavirin and interferon. See, e.g., Beremguer et al. (1998) Proc. Assoc. Am. Physicians 110(2):98-112. These therapies suffer from a low sustained response rate and frequent side effects. See, e.g., Hoofnagle et al. (1997) N. Engl. J. Med. 336:347. Currently, no vaccine is available for HCV infection.

Pending patent applications, Serial No. 60/194,607, filed April 5, 2000, and Serial No. 60/198,204, filed April 19, 2000, both having common ownership with the present application, disclose certain macrocyclic NS-3 serine protease inhibitors of hepatitis C virus.

There is a need for new treatments and therapies for HCV infection. It is, therefore, an object of this invention to provide compounds useful in the treatment or prevention or amelioration of one or more symptoms of hepatitis C.

It is a further object herein to provide methods of treatment or prevention or amelioration of one or more symptoms of hepatitis C.

A still further object of the present invention is to provide methods for modulating the activity of serine proteases, particularly the HCV NS3/NS4a serine protease, using the compounds provided herein.

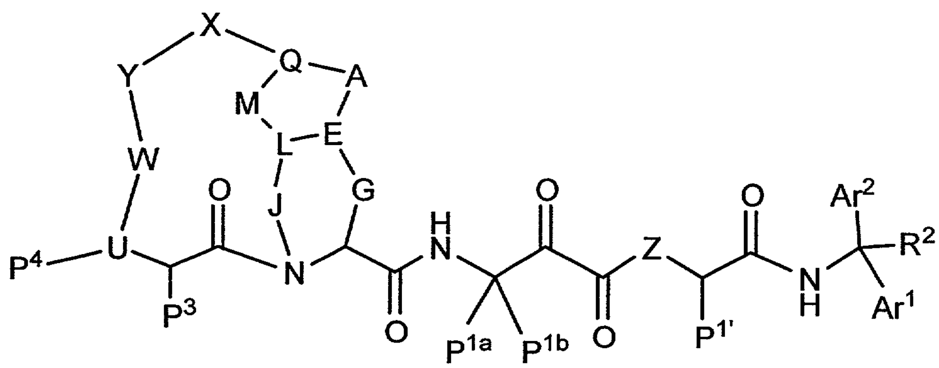
Another object herein is to provide methods of modulating the processing of the HCV polypeptide using the compounds provided herein.

SUMMARY OF THE INVENTION

In its many embodiments, the present invention provides a novel class of inhibitors of the HCV protease, pharmaceutical compositions containing one or more of the compounds, methods of preparing pharmaceutical formulations comprising one or more such compounds, and methods of treatment, prevention or amelioration or one or more of the symptoms of hepatitis C. Also provided are methods of modulating the interaction of an HCV polypeptide with HCV protease. Among the compounds provided herein, compounds that inhibit HCV NS3/NS4a serine protease activity are preferred. The presently disclosed compounds generally contain about four or more amino acid residues and less than about twelve amino acid residues. Specifically, the present application discloses peptide compounds, defined further below in Formulae I, II and III.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

In its first embodiment, the present invention provides a compound of Formula I:



Formula I

wherein:

X and Y are independently selected from the moieties: alkyl, alkyl-aryl, heteroalkyl, heteroaryl, aryl-heteroaryl, alkyl-heteroaryl, cycloalkyl, alkyl ether, alkyl-aryl ether, aryl ether, alkyl amino, aryl amino, alkyl-aryl amino, alkyl thio, alkyl-aryl thio, aryl thio, alkyl sulfone, alkyl-aryl sulfone, aryl sulfone, alkyl-alkyl sulfoxide, alkyl-aryl sulfoxide, alkyl amide, alkyl-aryl amide, aryl amide, alkyl sulfonamide, alkyl-aryl sulfonamide, aryl sulfonamide, alkyl urea, alkyl-aryl urea, aryl urea, alkyl carbamate, alkyl-aryl carbamate, aryl carbamate, alkyl-hydrazide, alkyl-aryl hydrazide, alkyl hydroxamide, alkyl-aryl hydroxamide, alkyl sulfonyl, aryl sulfonyl, heteroalkyl sulfonyl, heteroaryl sulfonyl, alkyl carbonyl, aryl carbonyl, heteroalkyl carbonyl, heteroaryl carbonyl, alkoxycarbonyl, aryloxycarbonyl, heteroaryloxycarbonyl, alkylaminocarbonyl, arylaminocarbonyl, heteroarylaminocarbonyl or a combination thereof, with the proviso that X and Y may optionally be additionally substituted with X^{11} or $X^{12;}$

X^{11} is alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkyl-alkyl, heterocyclyl, heterocyclylalkyl, aryl, alkylaryl, arylalkyl, heteroaryl, alkylheteroaryl, or heteroarylalkyl, with the proviso that X^{11} may be additionally optionally substituted with X^{12};

X^{12} is hydroxy, alkoxy, aryloxy, thio, alkylthio, arylthio, amino, alkylamino, arylamino, alkylsulfonyl, arylsulfonyl, alkylsulfonamido, arylsulfonamido, carboxy, carbalkoxy, carboxamido, alkoxycarbonylamino, alkoxycarbonyloxy, alkylureido, arylureido, halogen, cyano, or nitro, with the proviso that said alkyl, alkoxy, and aryl may be additionally optionally substituted with moieties independently selected from X^{12};

W may be present or absent, and if W is present, W is selected form C=O, C=S, or SO_2;

Q may be present or absent, and when Q is present, Q is CH, N, P, $(CH_2)_p$, $(CHR)_p$, $(CRR')_p$, O, RNR, S, or SO_2; and when Q is absent, M is also absent, A is directly linked to X;

A is O, CH_2, $(CHR)_p$, $(CHR\text{-}CHR')_p$, $(CRR')_p$, NR, S, SO_2 or a bond;

U is selected form O, N, or CH;

E is CH, N or CR, or a double bond towards A, L or G;

G may be present or absent, and when G is present, G is $(CH_2)_p$, $(CHR)_p$, or $(CRR')_p$; and when G is absent, J is present and E is directly connected to the carbon atom where G was connected to;

J may be absent or present, and when J is present, J is $(CH_2)_p$, $(CHR)_p$, or $(CRR')_p$, SO_2, NH, NR or O; and when J is absent, G is present and L is directly linked to nitrogen;

L may be present or absent, and when L is present, L is CH, CR, O, S or NR; and when L is absent, then M may be absent or present, and if M is present with L being absent, then M is directly and independently linked to E, and J is directly and independently linked to E;

M may be present or absent, and when M is present, M is O, NR, S, SO_2, $(CH_2)_p$, $(CHR)_p$, $(CHR\text{-}CHR')_p$, or $(CRR')_p$;

p is a number from 0 to 6;

R and R' are independently selected from the group consisting of H; C1-C10 alkyl; C2-C10 alkenyl; C3- C8 cycloalkyl; C3-C8 heterocycloalkyl, alkoxy, aryloxy, alkylthio, arylthio, amino, amido, cyano, nitro; (cycloalkyl)–alkyl and (heterocycloalkyl)alkyl, wherein said cycloalkyl is made of three to eight carbon atoms, and zero to six oxygen, nitrogen, sulfur, or phosphorus atoms, and said alkyl is of one to six carbon atoms; aryl; heteroaryl; alkyl-aryl; and alkyl-heteroaryl;

with said alkyl, heteroalkyl, alkenyl, heteroalkenyl, aryl, heteroaryl, cycloalkyl and heterocycloalkyl moieties may be optionally substituted, with said term "substituted" referring to optional and suitable substitution with one or more moieties selected from the group consisting of alkyl, alkenyl, alkynyl, aryl, aralkyl, cycloalkyl, heterocyclic, halogen, hydroxy, thio, alkoxy, aryloxy, alkylthio, arylthio, amino, amido, cyano, nitro, sulfonamido; and

P^{1a}, P^{1b}, $P^{1'}$ and P^3 are independently selected from:

H, C1-C10 straight or branched chain alkyl, C2-C10 straight or branched chain alkenyl, and C3-C8 cycloalkyl, C3-C8 heterocyclic; (cycloalkyl)alkyl or (heterocyclyl)alkyl , wherein said cycloalkyl is made up of 3 to 8 carbon atoms, and zero to 6 oxygen, nitrogen, sulfur, or phosphorus atoms, and said alkyl is of 1 to 6 carbon atoms;

aryl, heteroaryl, arylalkyl, or heteroarylalkyl, wherein said alkyl is of 1 to 6 carbon atoms;

wherein said alkyl, alkenyl, cycloalkyl, heterocyclyl; (cycloalkyl)alkyl and (heterocyclyl)alkyl moieties may be optionally substituted with R", and further wherein said P^{1a} and P^{1b} may optionally be joined to each other to form a spirocyclic or spiroheterocyclic ring, with said spirocyclic or spiroheterocyclic ring containing zero to six oxygen, nitrogen, sulfur, or phosphorus atoms, and may be additionally optionally substituted with R";

R" is hydroxy, alkoxy, aryloxy, thio, alkylthio, arylthio, amino, alkylamino, arylamino, alkylsulfonyl, arylsulfonyl, alkylsulfonamido, arylsulfonamido, carboxy, carbalkoxy, carboxamido, alkoxycarbonylamino, alkoxycarbonyloxy, alkylureido, arylureido, halogen, cyano, or nitro moiety, with the proviso that the alkyl, alkoxy, and aryl may be additionally optionally substituted with moieties independently selected from R";

Z is O, NH or NR'";

R'" is alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkyl-alkyl, heterocyclyl, heterocyclylalkyl, aryl, alkylaryl, arylalkyl, heteroaryl, alkylheteroaryl, or heteroarylalkyl moiety, with the proviso that R'" may be additionally optionally substituted with R";

Ar^1 and Ar^2 are independently selected from phenyl; 2-pyridyl, 3-pyridyl, 4-pyridyl or their corresponding N-oxides; 2-thiophenyl; 3-thiophenyl; 2-furanyl; 3-furanyl; 2-pyrrolyl; 3-pyrrolyl; 2-imidazolyl; 3(4)-imidazolyl; 3-(1,2,4-triazolyl); 5-tetrazolyl; 2-thiazolyl; 4-thiazolyl; 2-oxazolyl; or 4-oxazolyl; either or both of which may be optionally substituted with R^1;

R^1 is H, halogen, cyano, nitro, CF_3, $Si(alkyl)_3$, straight-chain or branched lower alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkyl-alkyl, aryl, alkylaryl, arylalkyl, heteroaryl, hydroxy, alkoxy, aryloxy, alkoxycarbonyloxy, (alkylamino)carbonyloxy, mercapto, alkylthio, arylthio, alkylsulfinyl, heterocyclylsulfinyl, arylsulfinyl, heteroarylsulfinyl, alkylsulfonyl, heterocyclylsulfonyl, arylsulfonyl, heteroarylsulfonyl, alkylcarbonyl, arylcarbonyl, carboxy, alkoxycarbonyl, aryloxycarbonyl, heteroaryloxycarbonyl, alkyaminocarbonyl, arylaminocarbonyl, amino, alkylamino, arylamino, alkylsulfonamide, arylsulfonamide, alkoxycarbonbylamino, alkylureido, or arylureido;

P^4 is H, linear or branched alkyl, arylalkyl or aryl; and

$R^{2'}$ is H, cyano, CF_3, straight-chain or branched lower alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkyl-alkyl, aryl, alkylaryl, arylalkyl, heteroaryl, alkylsulfonyl, arylsulfonyl, carboxy, alkoxycarbonyl, aryloxycarbonyl, alkyaminocarbonyl, (allylamino)carbonyl), or arylaminocarbonyl.

Suitably, $R^{2'}$ is selected from the group consisting of H, alkyl, alkenyl, alkoxycarbonyl, or (allylamino) carbonyl and preferably wherein $R^{2'}$ is H, U is N and P^4 is H.

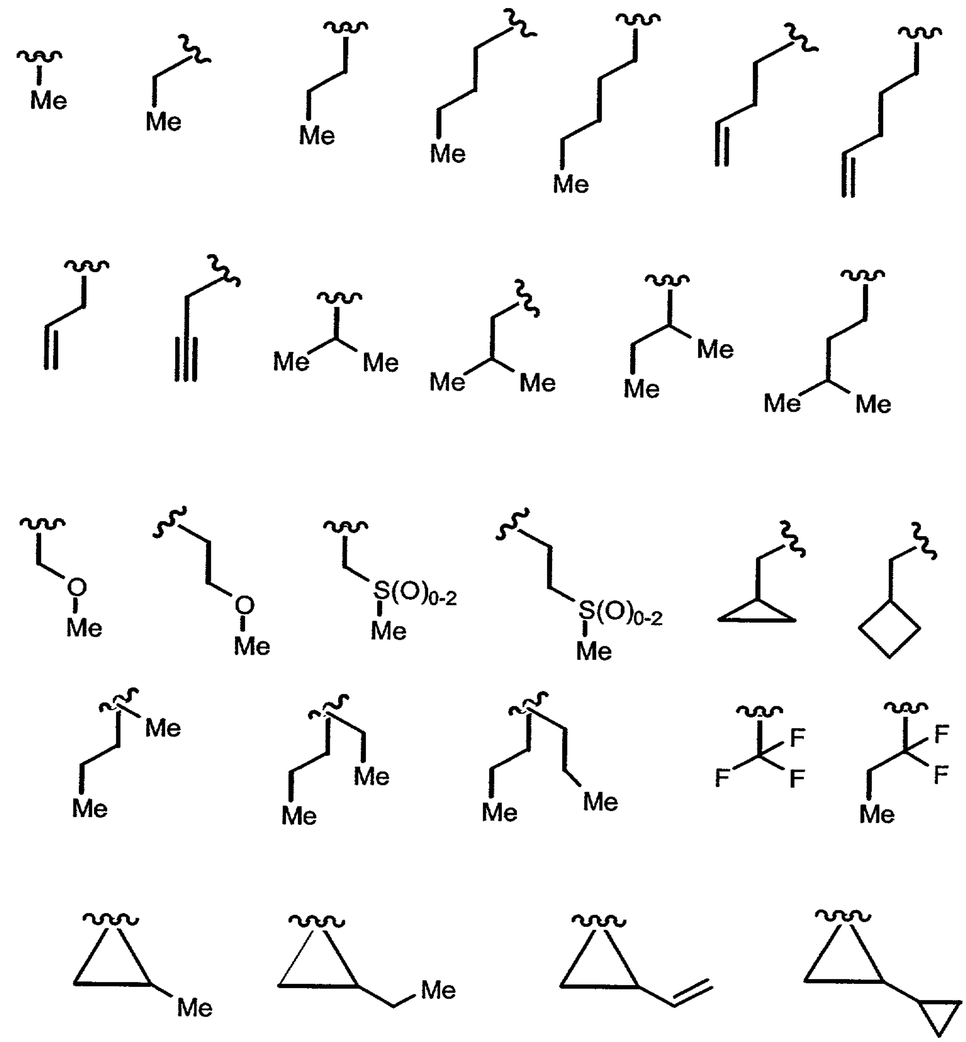
Advantageously, Ar^1 and Ar^2 are independently selected from the group consisting of phenyl, 2-thiophenyl, 2-furanyl, 3-furanyl, 3(4)-imidazolyl, 3-(1,2,4-triazolyl), 5-tetrazolyl, or 2-thiazolyl, preferably Ar^2 is phenyl and Ar^1 is selected from the group consisting of 3-(1,2,4-triazolyl),5-tetrazolyl, or 2-thiazolyl and U is N and P^4 is H.

Suitably, R^1 is H, CF_3, CH_3, alkyl or alkenyl.

Usually, $P1'$ is either H or CH_3.

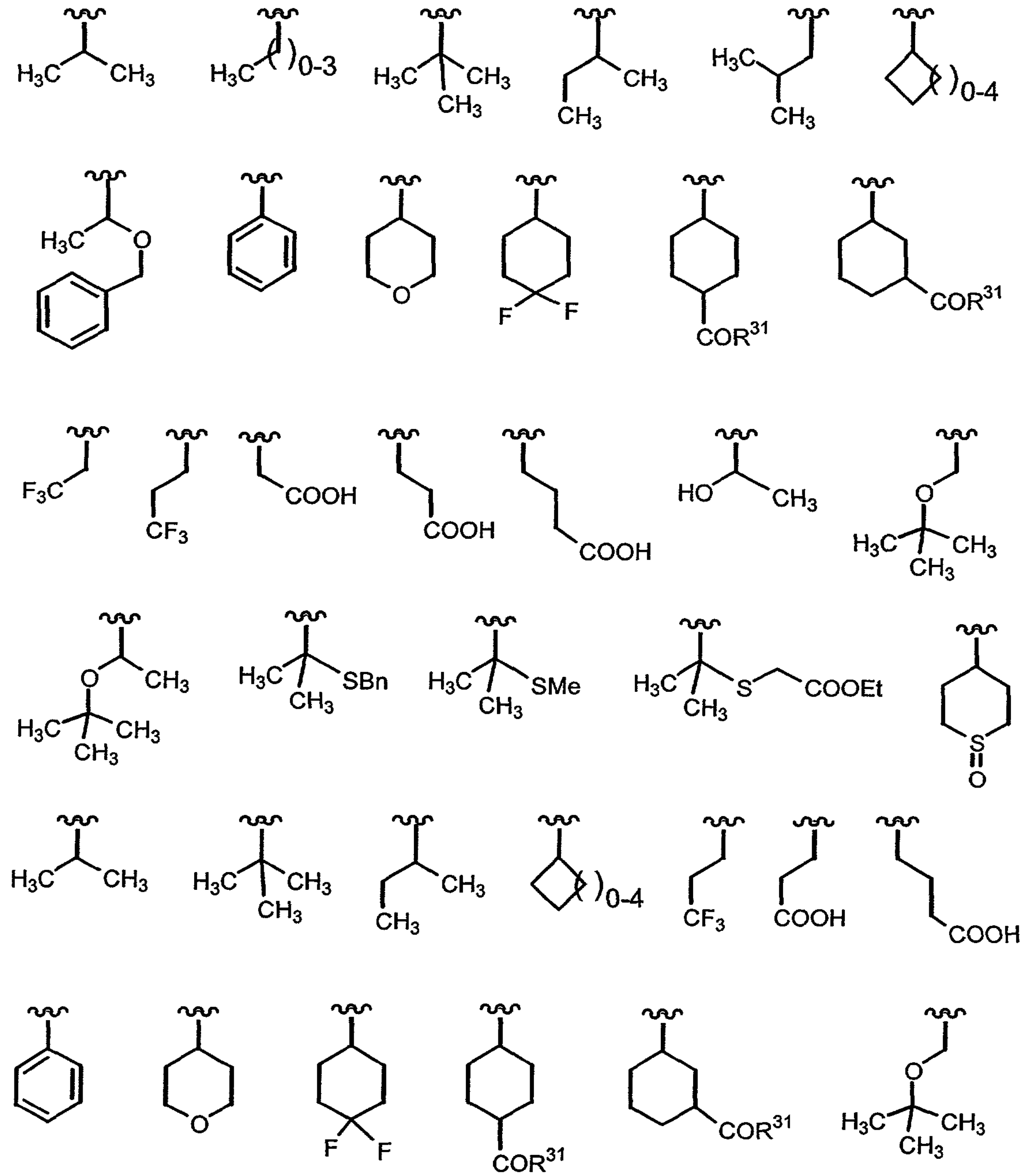
Suitably, when $P^{1'}$ is H then $P^{1'}$ and the adjacent nitrogen and carbonyl moieties correspond to the residuum of a glycine unit.

Preferably, P^{1a} and P^{1b} are independently selected from the group consisting of the following moieties:



Advantageously, U is N and P^4 is H and Z is NH.

Suitably, P^3 is selected from the group consisting of:

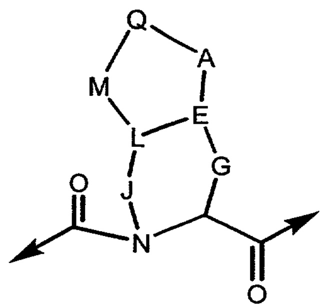


wherein R^{31} = OH or O-alkyl.

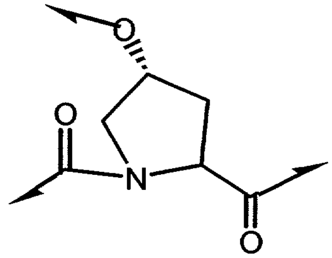
Suitably, P^4 is selected from the group consisting of H, tertiary butyl, isobutyl and phenyl substituents.

Suitably, Z is NH and U is N and P^3 is as set forth above.

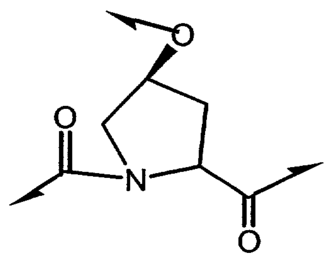
In another suitable expression of Formula I, the moiety:



is

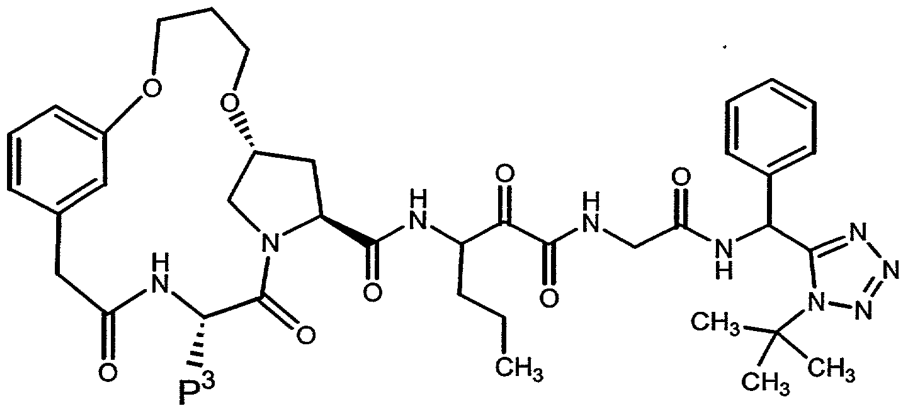


or

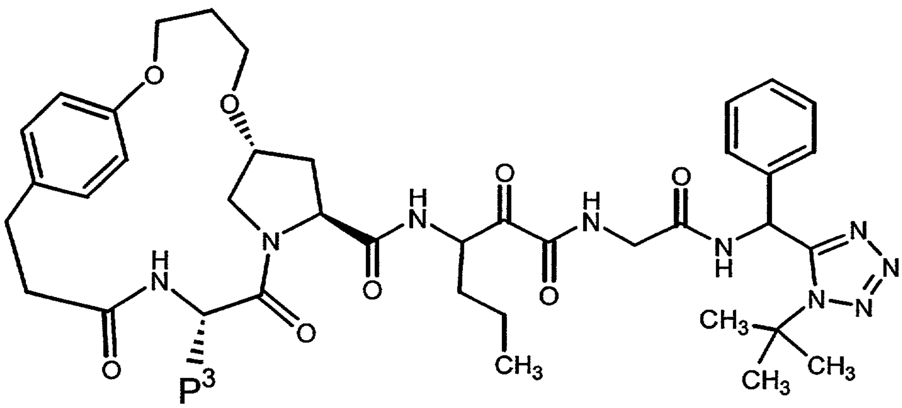


Suitably, Z is NH and U is N.

The compound of Formula I, wherein said compound is selected from the group consisting of compounds having the structural formulae:

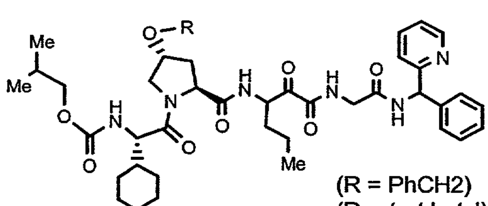


or

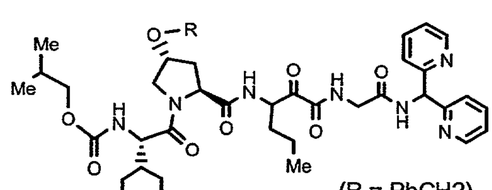


wherein P^3 is an isopropyl, tertiary butyl, cyclopentyl, or cyclohexyl moiety.

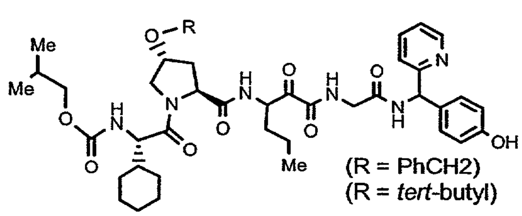
A preferred compound of Formula I exhibiting HCV protease inhibitory activity, including enantiomers, stereoisomers and tautomers of said compound, and pharmaceutically acceptable salts or solvates of said compound, said compound being selected from the compounds of structures listed below:



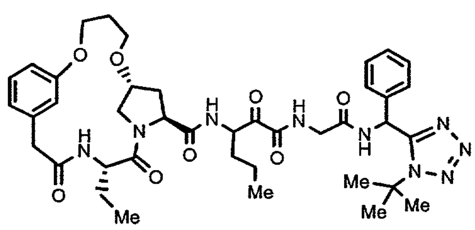
(R = PhCH2)
(R = *tert*-butyl)

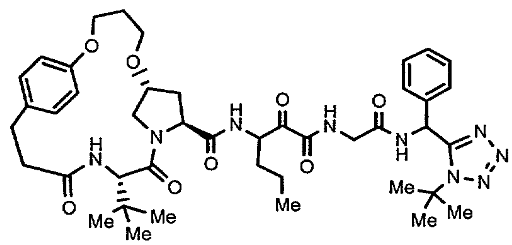


(R = PhCH2)
(R = *tert*-butyl)



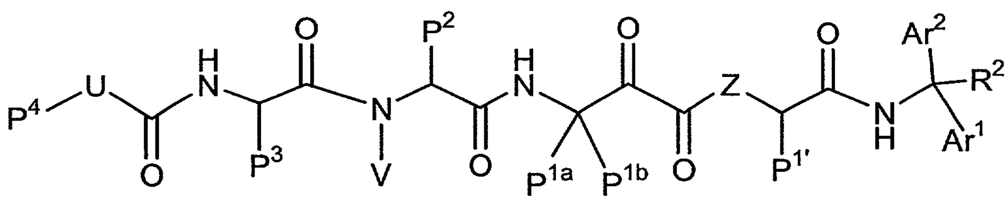
(R = PhCH2)
(R = *tert*-butyl)





In one embodiment, the present invention discloses compounds of Formula I as inhibitors of HCV protease, especially the HCV NS3/NS4a serine protease, or a pharmaceutically acceptable derivative thereof, where the various definitions are given above.

In another embodiment, the present invention discloses compounds including enantiomers, stereoisomers, rotomers and tautomers of said compound, and pharmaceutically acceptable salts, solvates or derivatives thereof, with said compound having the general structure shown in Formula II:



Formula II

wherein:

P^{1a}, P^{1b}, $P^{1'}$, P^2, and P^3 are independently:

H, C1-C10 straight or branched chain alkyl, C2-C10 straight or branched chain alkenyl, and C3-C8 cycloalkyl, C3-C8 heterocyclic; (cycloalkyl)alkyl or (heterocyclyl)alkyl , wherein said cycloalkyl is made up of 3 to 8 carbon atoms, and zero to 6 oxygen, nitrogen, sulfur, or phosphorus atoms, and said alkyl is of 1 to 6 carbon atoms;

aryl, heteroaryl, arylalkyl, or heteroarylalkyl, wherein said alkyl is of 1 to 6 carbon atoms;

wherein said alkyl, alkenyl, cycloalkyl, heterocyclyl; (cycloalkyl)alkyl and (heterocyclyl)alkyl moieties may be optionally substituted with R", and further wherein said P^{1a} and P^{1b} may optionally be joined to each other to form a spirocyclic or spiroheterocyclic ring, with said spirocyclic or spiroheterocyclic ring

containing zero to six oxygen, nitrogen, sulfur, or phosphorus atoms, and may be additionally optionally substituted with R";

R" is hydroxy, alkoxy, aryloxy, thio, alkylthio, arylthio, amino, alkylamino, arylamino, alkylsulfonyl, arylsulfonyl, alkylsulfonamido, arylsulfonamido, carboxy, carbalkoxy, carboxamido, alkoxycarbonylamino, alkoxycarbonyloxy, alkylureido, arylureido, halogen, cyano, or nitro moiety, with the proviso that the alkyl, alkoxy, and aryl may be additionally optionally substituted with moieties independently selected from R";

Z is O, NH or NR'";

R'" is alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkyl-alkyl, heterocyclyl, heterocyclylalkyl, aryl, alkylaryl, arylalkyl, heteroaryl, alkylheteroaryl, or heteroarylalkyl moiety, with the proviso that R'" may be additionally optionally substituted with R";

Ar^1 and Ar^2 are independently selected from phenyl; 2-pyridyl, 3-pyridyl, 4-pyridyl or their corresponding N-oxides; 2-thiophenyl; 3-thiophenyl; 2-furanyl; 3-furanyl; 2-pyrrolyl; 3-pyrrolyl; 2-imidazolyl; 3(4)-imidazolyl; 3-(1,2,4-triazolyl); 5-tetrazolyl; 2-thiazolyl; 4-thiazolyl; 2-oxazolyl; or 4-oxazolyl; either or both of which may be optionally substituted with R^1;

R^1 is H, halogen, cyano, nitro, CF_3, $Si(alkyl)_3$, straight-chain or branched lower alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkyl-alkyl, aryl, alkylaryl, arylalkyl, heteroaryl, hydroxy, alkoxy, aryloxy, alkoxycarbonyloxy, (alkylamino)carbonyloxy, mercapto, alkylthio, arylthio, alkylsulfinyl, heterocyclylsulfinyl, arylsulfinyl, heteroarylsulfinyl, alkylsulfonyl, heterocyclylsulfonyl, arylsulfonyl, heteroarylsulfonyl, alkylcarbonyl, arylcarbonyl, carboxy, alkoxycarbonyl, aryloxycarbonyl, heteroaryloxycarbonyl, alkyaminocarbonyl, arylaminocarbonyl, amino, alkylamino, arylamino, alkylsulfonamide, arylsulfonamide, alkoxycarbonbylamino, alkylureido, or arylureido;

P^4 is H, linear or branched alkyl, arylalkyl or aryl;

$R^{2'}$ is H, cyano, CF_3, straight-chain or branched lower alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkyl-alkyl, aryl, alkylaryl, arylalkyl, heteroaryl, alkylsulfonyl, arylsulfonyl, carboxy, alkoxycarbonyl, aryloxycarbonyl, alkyaminocarbonyl, or arylaminocarbonyl;

U is O, NH, CH_2 or CHR"; and

V is H, methyl, or lower alkyl.

In a suitable formulation in Formula II, R2' is selected from the group consisting of H, alkyl, alkenyl,, alkoxycarbonyl, or (allylamino) carbonyl.

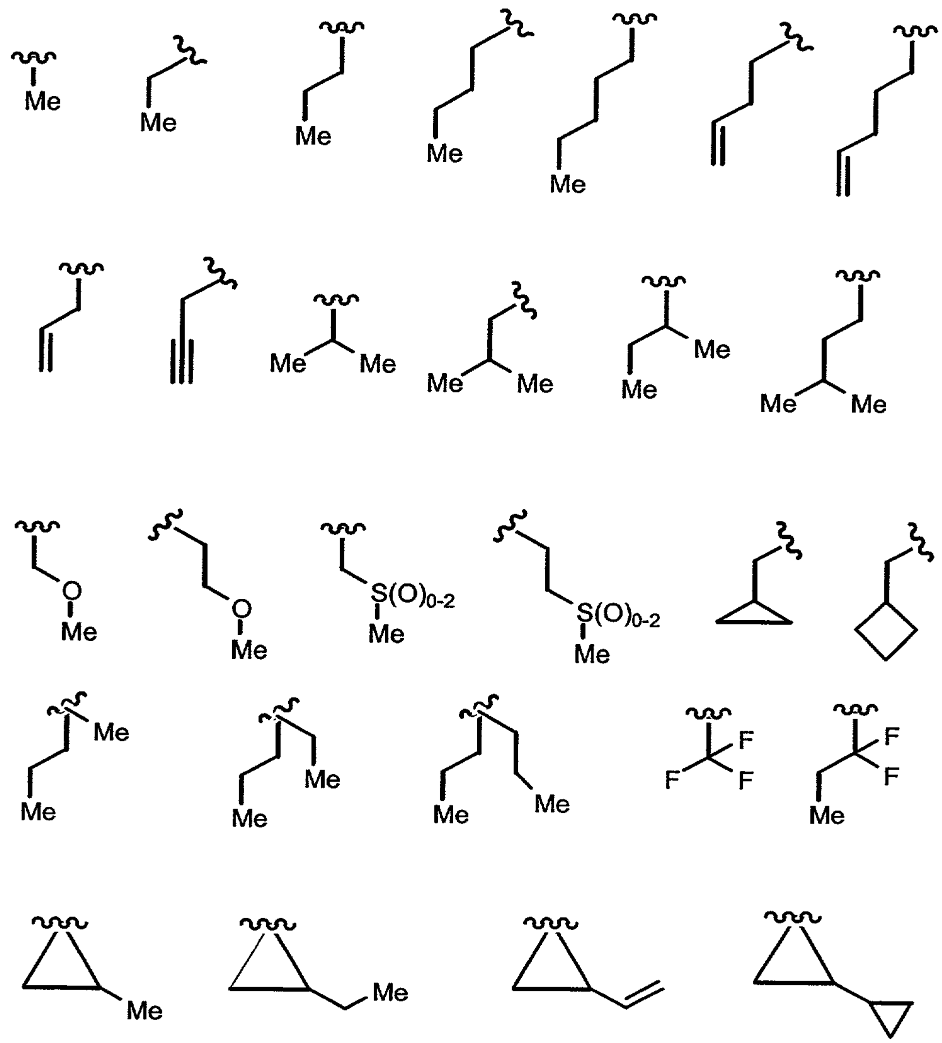
Advantageously in Formula II, Ar^1 and Ar^2 are independently selected from the group consisting of phenyl, 2-thiophenyl, 2-furanyl, 3-furanyl, 3(4)-imidazolyl, 3-(1,2,4-triazolyl), 5-tetrazolyl, or 2-thiazolyl.

Preferably, Ar^2 is phenyl and Ar^1 is selected from the group consisting of 3-(1,2,4-triazolyl),5-tetrazolyl, or 2-thiazolyl.

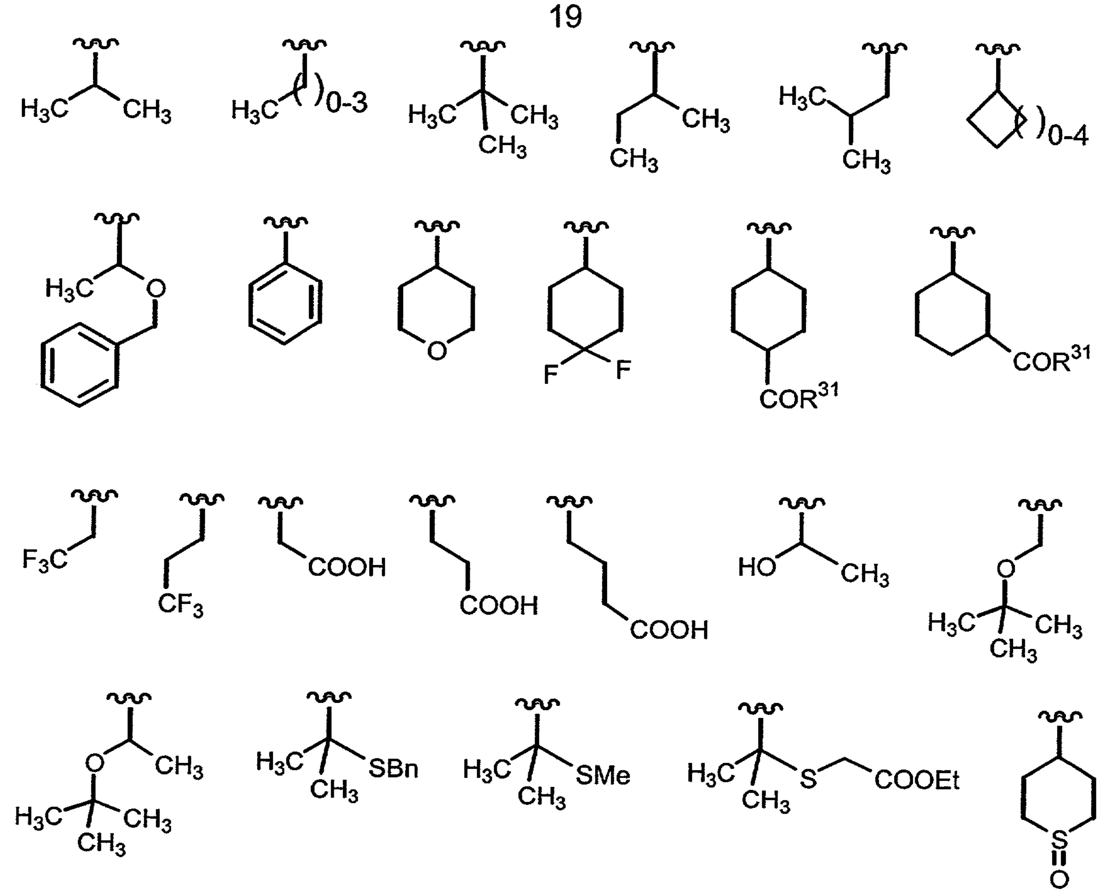
Suitably in Formula II, R^1 is H, CF_3, CH_3, alkyl or alkenyl and $P^{1'}$ is either H or CH_3.

Advantageously, $P^{1'}$ is H such that $P^{1'}$ and the adjacent nitrogen and carbonyl moieties correspond to the residuum of glycine unit.

Suitably in Formula II, P^{1a} and P^{1b} is selected from the group consisting of the following moieties:

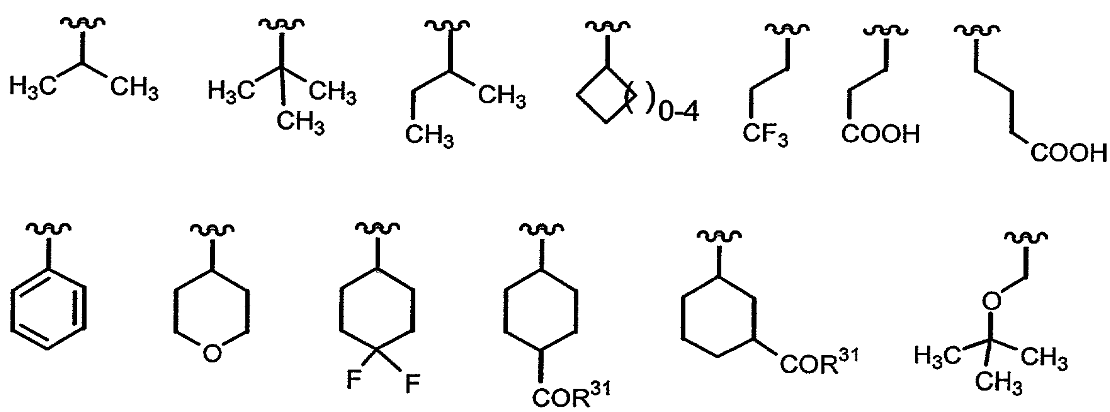


Advantageously in Formula II, P^3 is selected from the group consisting of:



wherein R^{31} = OH or O-alkyl.

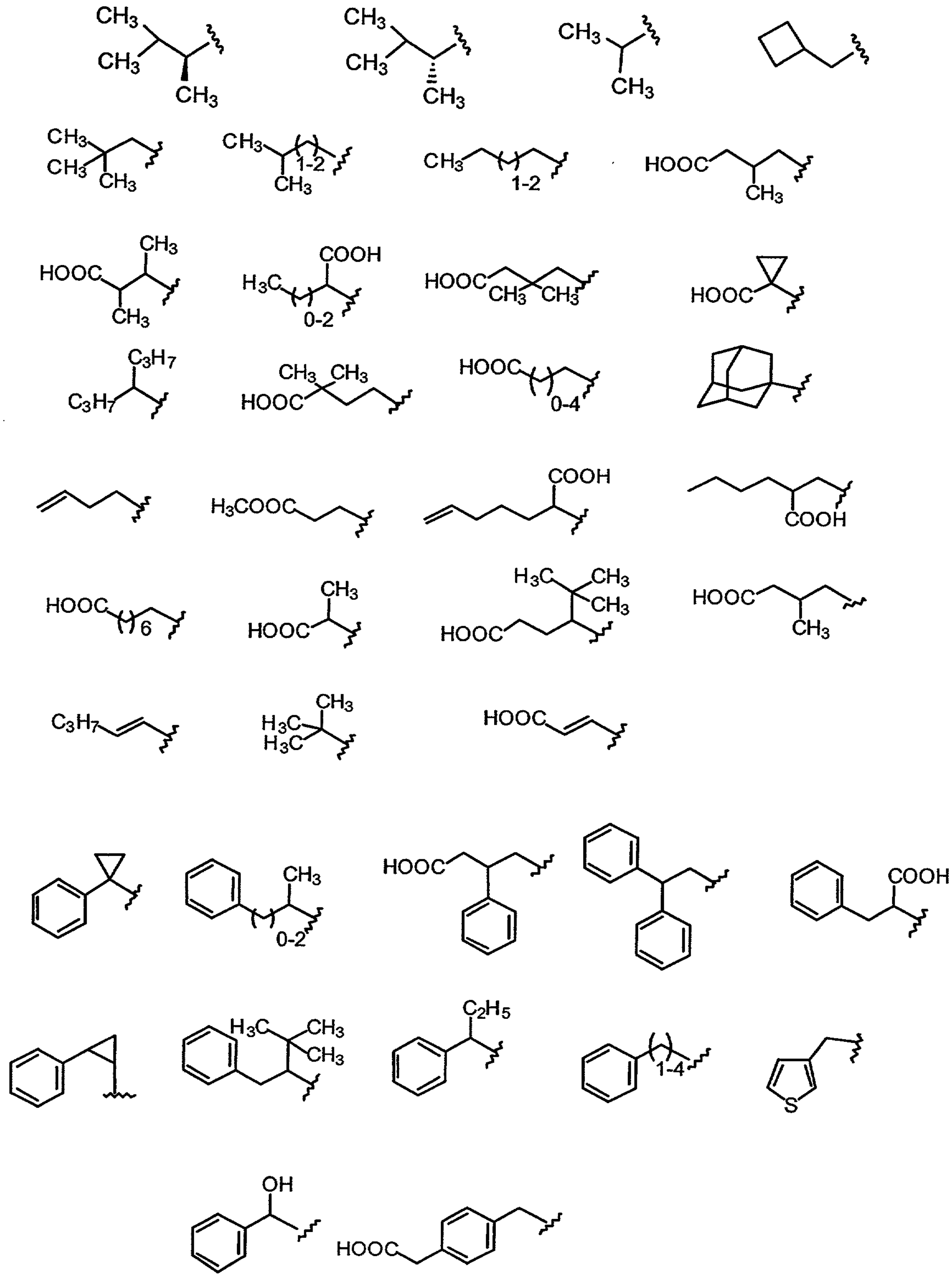
Preferably in Formula II, R^3 is selected from the group consisting of the following moieties:

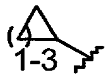
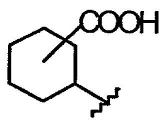
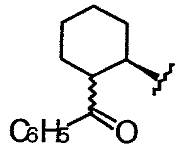
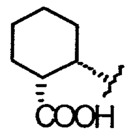
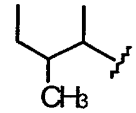
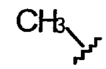


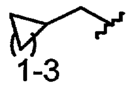
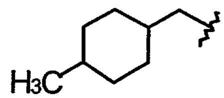
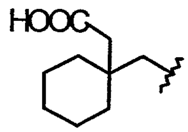
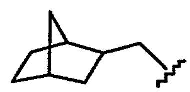
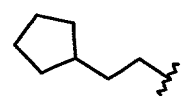
Suitably in Formula II, U is N and P^4 is alkyl or arylalkyl.

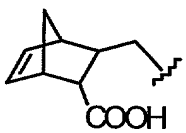
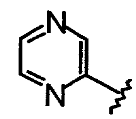
Preferably U is O or CH_2.

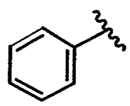
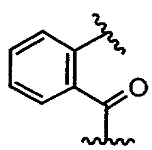
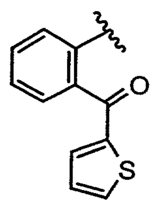
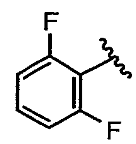
P^4 is selected from the following moieties:

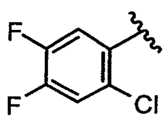
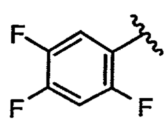
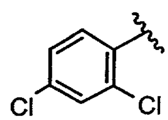
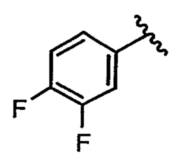
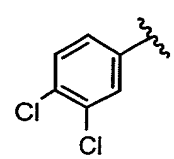


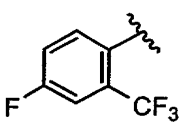
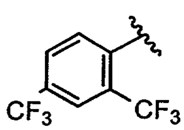
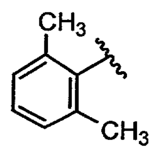
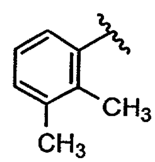
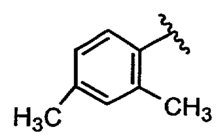







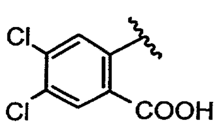
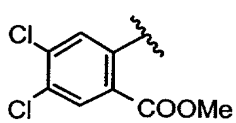
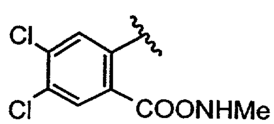
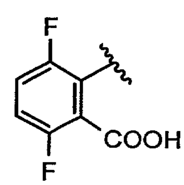






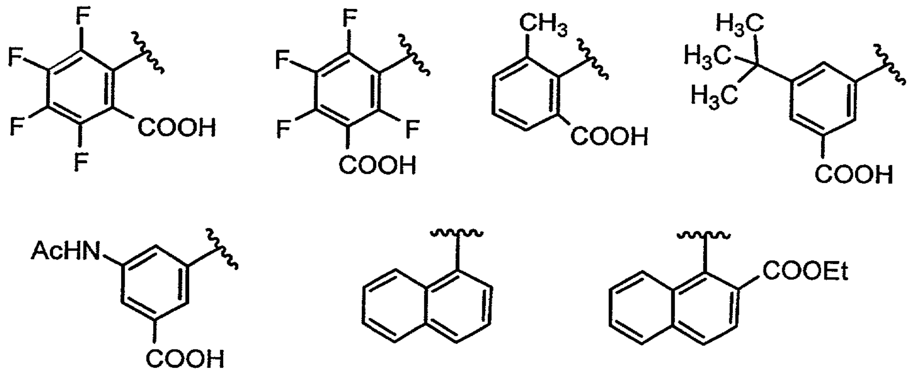



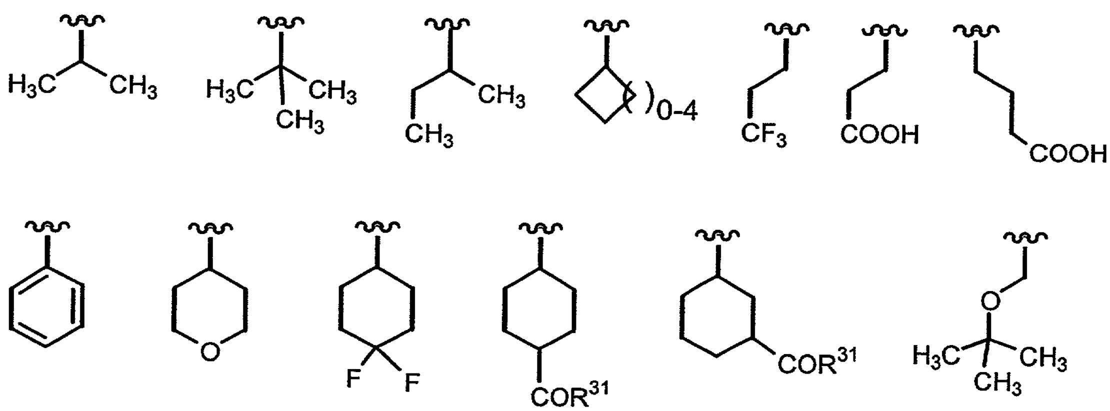







Suitably in Formula II, U is CH_2 and P^4 is phenyl or U is O and P^4 is selected from the group consisting of methyl, tertiary butyl, isobutyl, and 2,3-dimethylpropyl.

In Formula II, P^2 and P^3 are independently selected from the group consisting of: H, linear alkyl, branched alkyl, or arylalkyl, such that P^2 or P^3 and the adjacent nitrogen and carbonyl moieties thereto correspond to the residuum of an alpha amino acid.

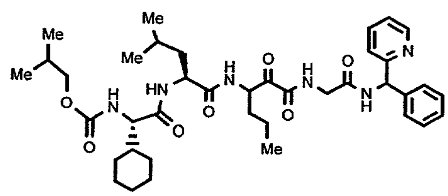
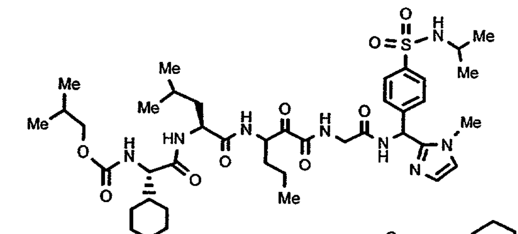
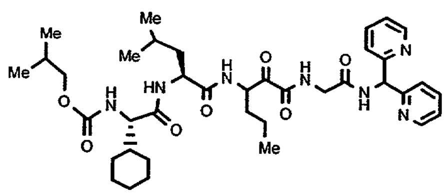
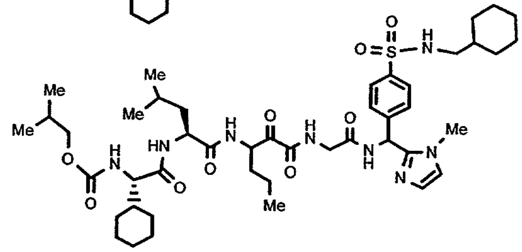
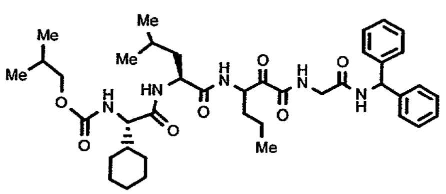
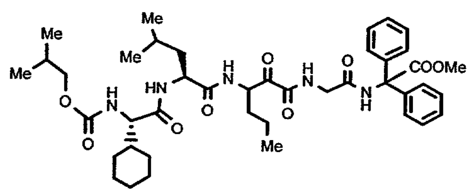
Preferably, P^3 is selected from the following moieties:



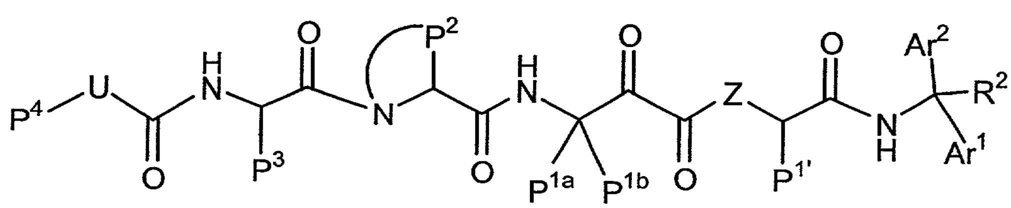
Suitably, P^3 is selected from the group consisting of isopropyl tertiary butyl, isobutyl and cyclohexyl substituents.

Advantageously, in Formula II, V is H.

A preferred compound of Formula II exhibiting HCV protease inhibitory activity, including enantiomers, stereoisomers and tautomers of said compound, and pharmaceutically acceptable salts or solvates of said compound, said compound being selected from the compounds of structures listed below:

In another embodiment, the present invention discloses compounds of Formula III as inhibitors of HCV protease, especially the HCV NS3/NS4a serine protease, or a pharmaceutically acceptable derivative thereof. The compound of Formula III has the following structure:



Formula III

wherein:

P^{1a}, P^{1b}, $P^{1'}$, P^2, and P^3 are independently selected from:

H, C1-C10 straight or branched chain alkyl, C2-C10 straight or branched chain alkenyl; and C3-C8 cycloalkyl, C3-C8 heterocyclic; (cycloalkyl)alkyl or (heterocyclyl)alkyl , wherein said cycloalkyl is made up of 3 to 8 carbon atoms, and zero to 6 oxygen, nitrogen, sulfur, or phosphorus atoms, and said alkyl is of 1 to 6 carbon atoms;

aryl, heteroaryl, arylalkyl, or heteroarylalkyl, wherein said alkyl is of 1 to 6 carbon atoms;

wherein said alkyl, alkenyl, cycloalkyl, heterocyclyl; (cycloalkyl)alkyl and (heterocyclyl)alkyl moieties may be optionally substituted with R", and further wherein said P^{1a} and P^{1b} may optionally be joined to each other to form a spirocyclic or spiroheterocyclic ring, with said spirocyclic or spiroheterocyclic ring containing zero to six oxygen, nitrogen, sulfur, or phosphorus atoms, and may be additionally optionally substituted with R";

R" is hydroxy, alkoxy, aryloxy, thio, alkylthio, arylthio, amino, alkylamino, arylamino, alkylsulfonyl, arylsulfonyl, alkylsulfonamido, arylsulfonamido, carboxy, carbalkoxy, carboxamido, alkoxycarbonylamino, alkoxycarbonyloxy, alkylureido, arylureido, halogen, cyano, or nitro moiety, with the proviso that the alkyl, alkoxy, and aryl may be additionally optionally substituted with moieties independently selected from R";

Z is O, NH or NR'";

R'" is alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkyl-alkyl, heterocyclyl, heterocyclylalkyl, aryl, alkylaryl, arylalkyl, heteroaryl, alkylheteroaryl, or heteroarylalkyl moiety, with the proviso that R'" may be additionally optionally substituted with R";

Ar1 and Ar2 are independently selected from phenyl; 2-pyridyl, 3-pyridyl, 4-pyridyl or their corresponding N-oxides; 2-thiophenyl; 3-thiophenyl; 2-furanyl; 3-furanyl; 2-pyrrolyl; 3-pyrrolyl; 2-imidazolyl; 3(4)-imidazolyl; 3-(1,2,4-triazolyl); 5-tetrazolyl; 2-thiazolyl; 4-thiazolyl; 2-oxazolyl; or 4-oxazolyl; either or both of which may be optionally substituted with R^1;

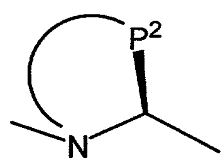
R^1 is H, halogen, cyano, nitro, CF_3, $Si(alkyl)_3$, straight-chain or branched lower alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkyl-alkyl, aryl, alkylaryl, arylalkyl, heteroaryl, hydroxy, alkoxy, aryloxy, alkoxycarbonyloxy, (alkylamino)carbonyloxy, mercapto, alkylthio, arylthio, alkylsulfinyl, heterocyclylsulfinyl, arylsulfinyl, heteroarylsulfinyl, alkylsulfonyl, heterocyclylsulfonyl, arylsulfonyl, heteroarylsulfonyl, alkylcarbonyl, arylcarbonyl, carboxy, alkoxycarbonyl, aryloxycarbonyl, heteroaryloxycarbonyl, alkyaminocarbonyl, arylaminocarbonyl, amino, alkylamino, arylamino, alkylsulfonamido, arylsulfonamido, alkoxycarbonbylamino, alkylureido, or arylureido;

P^4 is H, linear or branched alkyl, arylalkyl or aryl;

$R^{2'}$ is H, cyano, CF_3, straight-chain or branched lower alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkyl-alkyl, aryl, alkylaryl, arylalkyl, heteroaryl, alkylsulfonyl, arylsulfonyl, carboxy, alkoxycarbonyl, aryloxycarbonyl, alkyaminocarbonyl, or arylaminocarbonyl;

U is O, NH, CH_2 or CHR";

and

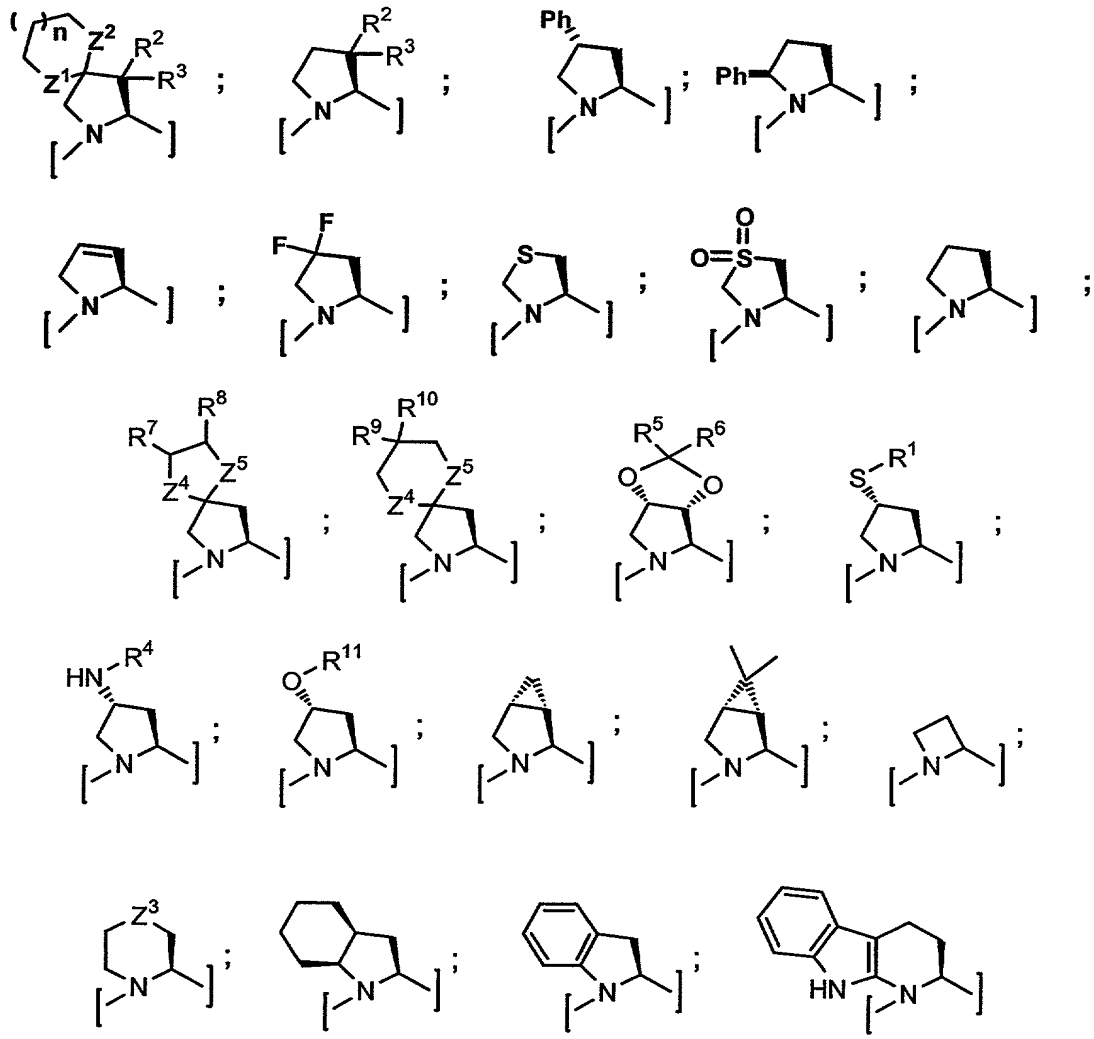


IV

where moiety IV indicates a cyclic ring structure, with the proviso that said cyclic ring structure does not contain a carbonyl group as part of the cyclic ring.

Preferably moiety IV is a five- or six-membered ring.

Advantageously, the moiety IV forms a structural unit selected from the group consisting of:



wherein n = 0, 1, 2, or 3; and

R^2 = **R^3** = H; **R^2** = C_1 to C_6 straight chainalkyl or cycloalkyl; **R^3** = H

R^4 = COAlkyl (straight chain or cyclic, C_1 to C_6); COAryl; COOAlkyl; COOAryl

R^5 = H; **R^6** = Alkyl (C_1 to C_3); **R^6** = H; **R^5** = Alkyl (C_1 to C_3)

R^7 = H; **R^8** = Alkyl (C_1 to C_3), CH_2OH; **R^8** = H; **R^7** = Alkyl (C_1 to C_3), CH_2OH;

R^9 = **R^{10}** = Alkyl (C_1 to C_3); **R^9** = H, **R^{10}** = Alkyl (C_1 to C_3), COOMe, COOH, CH_2OH;

R^{10} = H, R^9 = Alkyl (C_1 to C_3), COOMe, COOH, CH_2OH;

R^{11} = Alkyl (C_1 to C_6 straight chain, branched or cyclic), CH_2Aryl (may be substituted)

X^1 = H, Alkyl (C_1 to C_4, branched or straight chain); CH_2Aryl (substituted or unsubstituted)

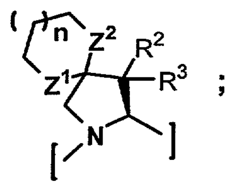
Z^1 = **Z^2** = S, O; **Z^1** = S, **Z^2** = O; **Z^1** = O, **Z^2** = S; **Z^1** = CH_2, **Z^2** = O; **Z^1** = O, **Z^2** = CH_2;

Z^1 = S, **Z^2** = CH_2; **Z^1** = CH_2, **Z^2** = S

Z^3 = CH_2, S, SO_2, NH, NR^4

$Z^4 = Z^5 = S, O$

Advantageously, the cyclic ring moiety is



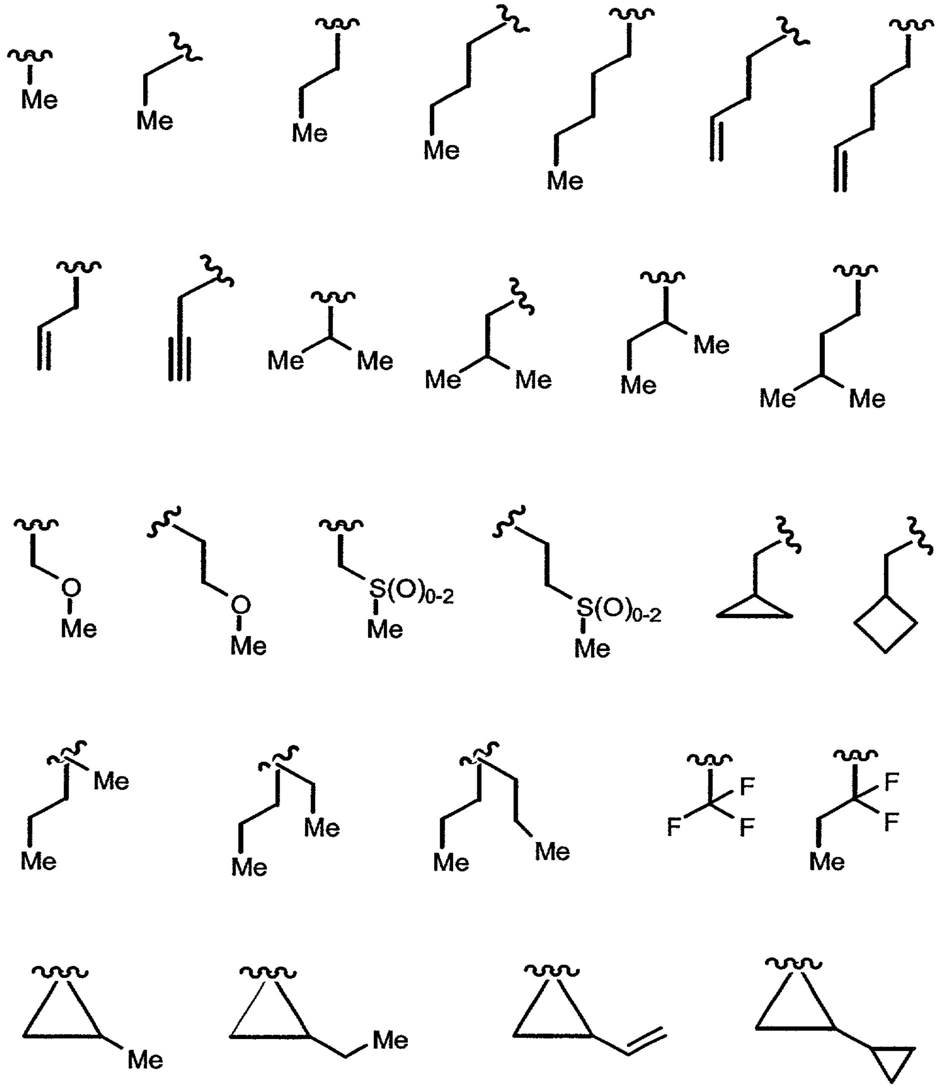
wherein Z^1 and Z^2 are S, R^2 and R^3 are H and n=1 or 2.

Suitably for the compound of Formula III, R2' is selected from the group consisting of H, alkyl, alkenyl, alkoxycarbonyl, or (allylamino) carbonyl and Ar^1 and Ar^2 are independently selected from the group consisting of phenyl, 2-thiophenyl, 2-furanyl, 3-furanyl, 3(4)-imidazolyl, 3-(1,2,4-triazolyl), 5-tetrazolyl, or 2-thiazolyl.

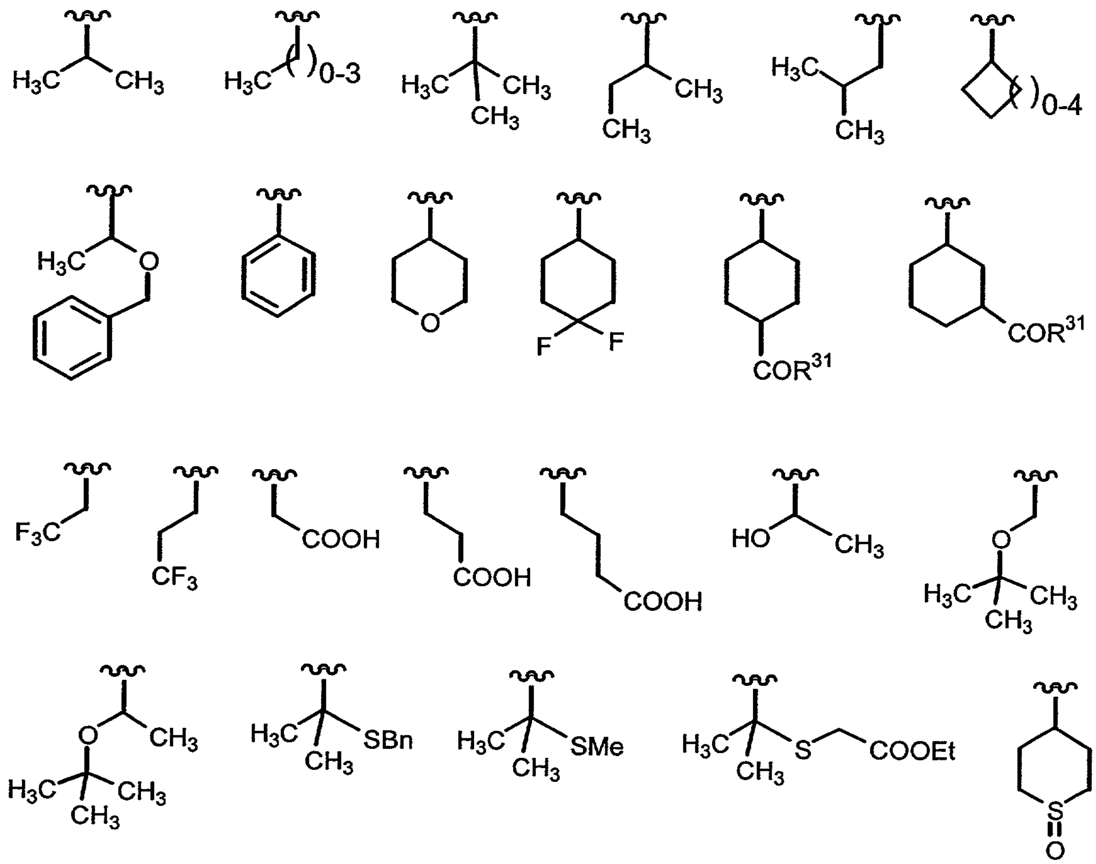
Advantageously, Ar^2 is phenyl and Ar^1 is selected from the group consisting of 3-(1,2,4-triazolyl),5-tetrazolyl, or 2-thiazolyl.

The compound of Formula III wherein in moiety IV, R^1 is H, CF_3, CH_3, alkyl or alkenyl and $P^{1'}$ is selected from the group consisting of H, F or CH_3. In another embodiment, $P^{1'}$ is H such that $P^{1'}$ and the adjacent nitrogen and carbonyl moieties correspond to the residuum of glycine unit.

The compound of Formula III, wherein P^{1a} and P^{1b} is selected from the group consisting of the following moieties:

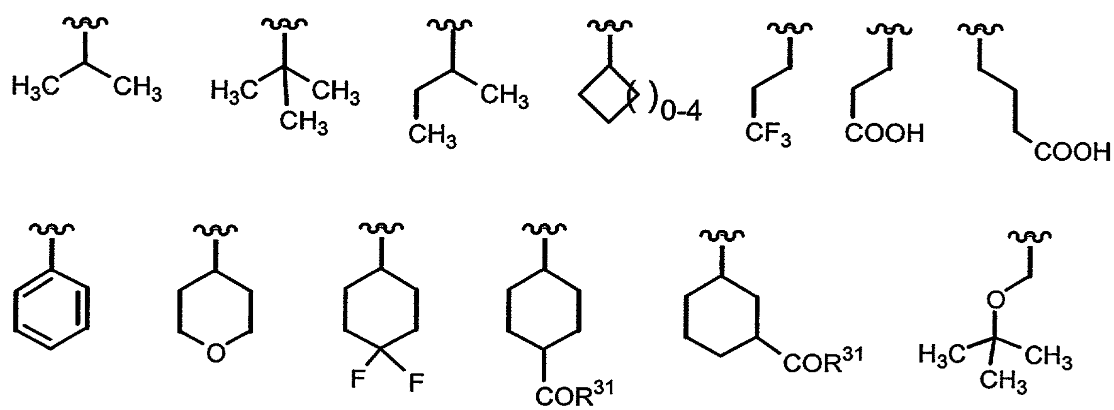


and P^3 is selected from the group consisting of:



wherein R^{31} = OH or O-alkyl.

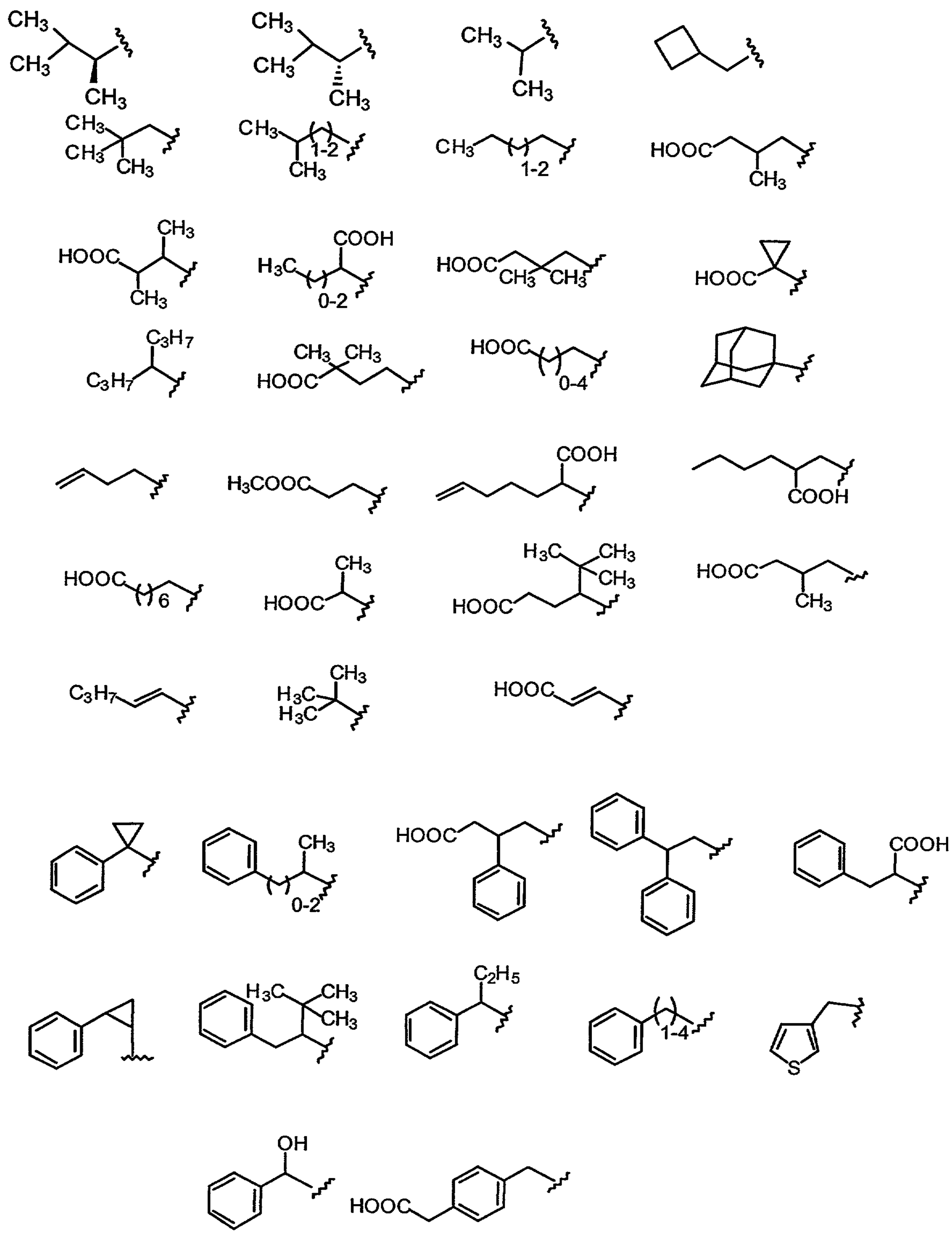
The compound of Formula III wherein moiety IV, R^3 is selected from the group consisting of the following moieties:

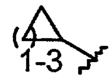
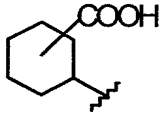
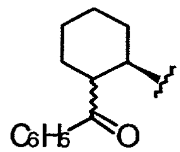
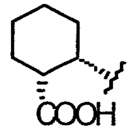
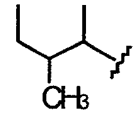
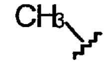


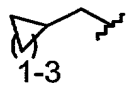
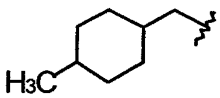
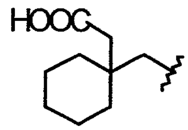
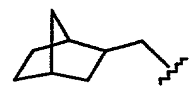
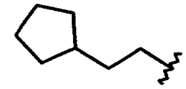
The compound of Formula III wherein moiety U is O or CH_2.

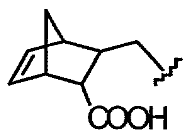
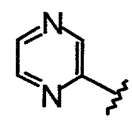
The compound of Formula III wherein in moiety IV, U is NH or O, and P^4 is alkyl or arylalkyl.

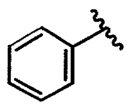
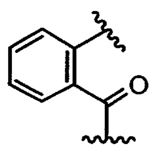
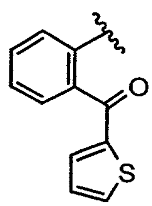
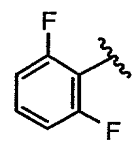
Advantageously moiety IV of Formula III comprises P^4 selected from the following moieties:

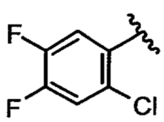
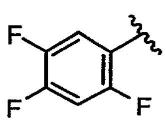
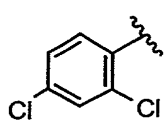
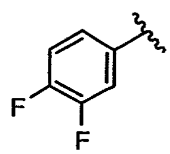
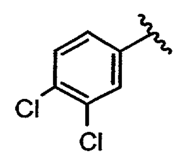


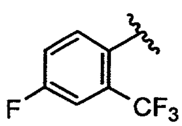
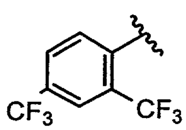
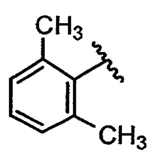
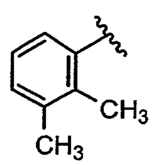
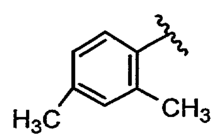
     

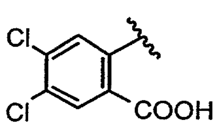
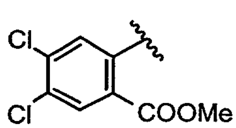
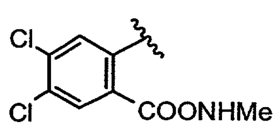
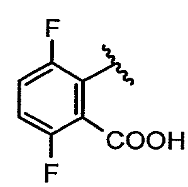
    

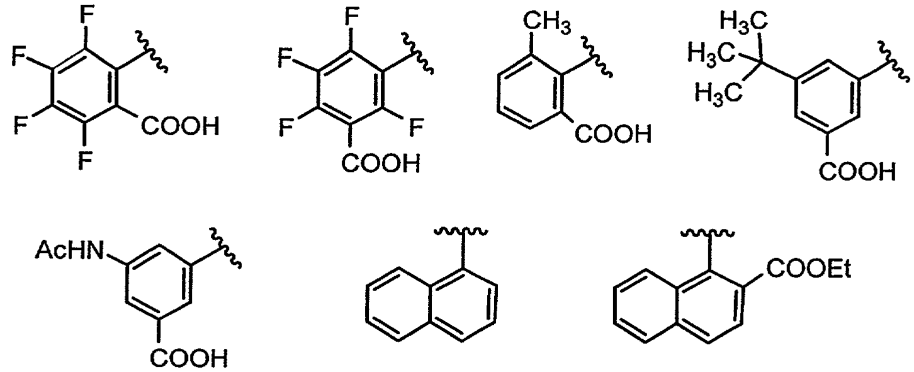
 

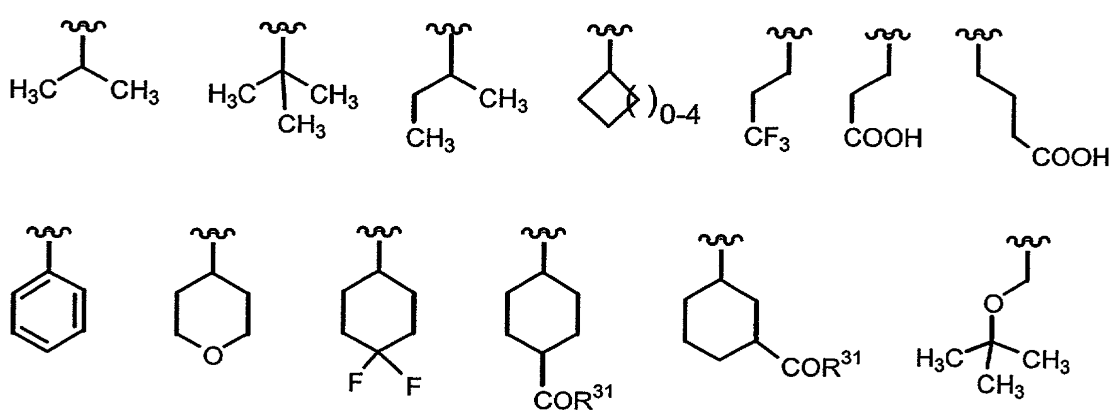
   



Advantageously in moiety IV, U is CH_2 and P^4 is phenyl or U is O and P^4 is selected from the group consisting of methyl, tertiary butyl, isobutyl, and 2,3-dimethylpropyl.

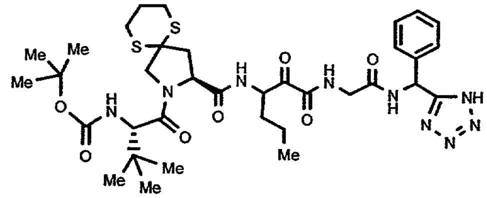
Suitably in moiety IV, P^2 and P^3 are independently selected from the group consisting of: H, linear alkyl, branched alkyl, or arylalkyl, such that P^2 OR P^3 and the adjacent nitrogen and carbonyl moieties thereto correspond to the residuum of an alpha amino acid.

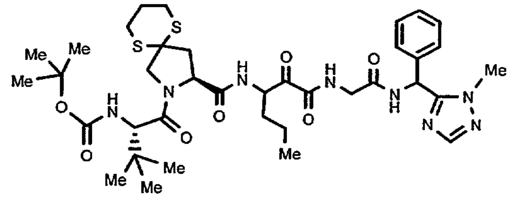
Advantageously, P^3 is selected from the following moieties:

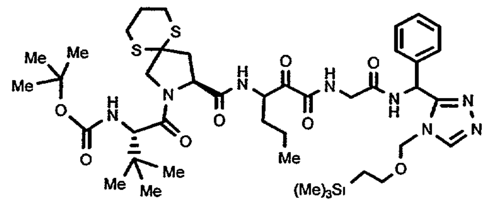


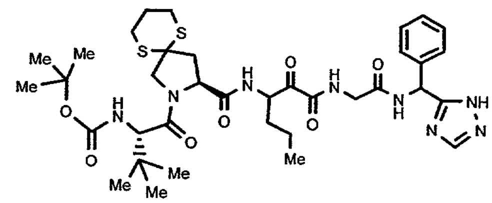
Preferably, P^3 is selected from the group consisting of isopropyl, tertiary butyl, isobutyl and cyclohexyl substituents.

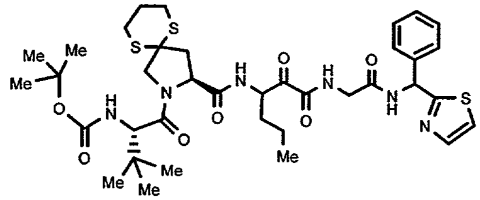
The compound according to Formula III, wherein said compound is selected from the group consisting of:

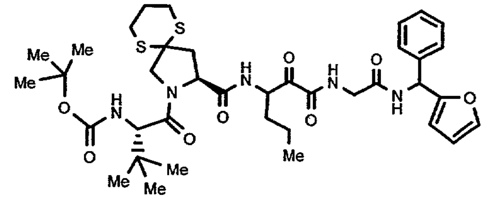


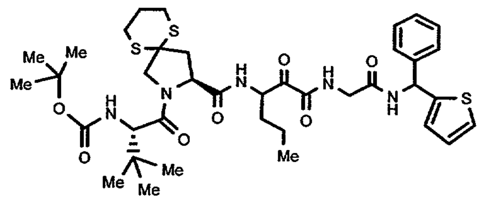


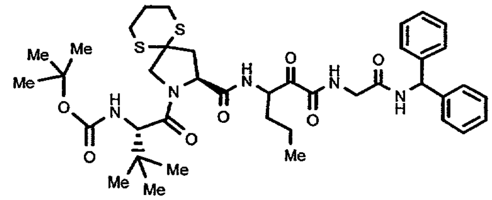


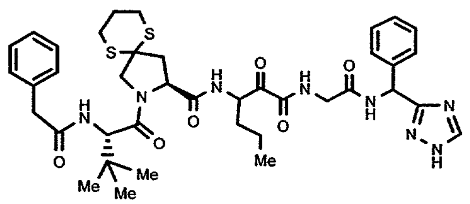


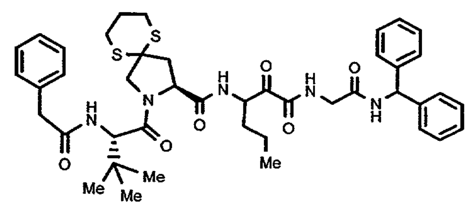


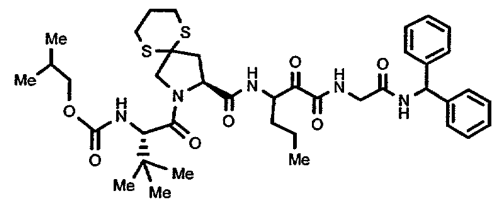


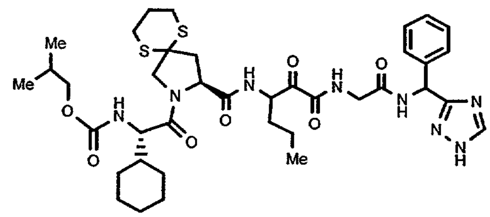


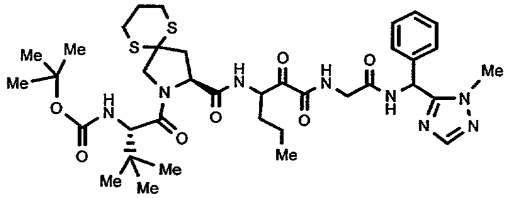


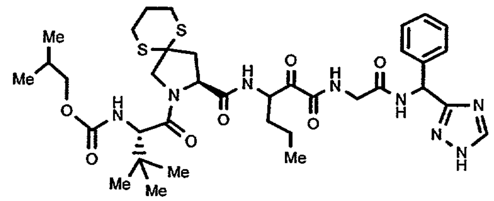


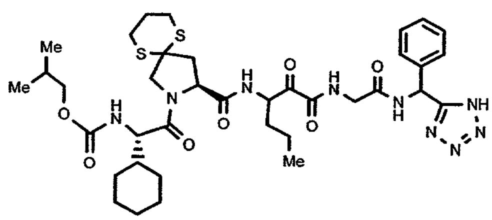


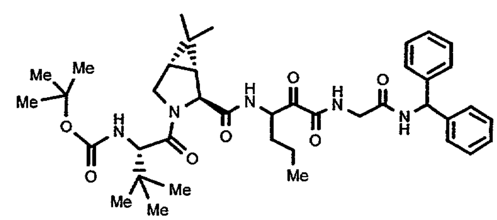


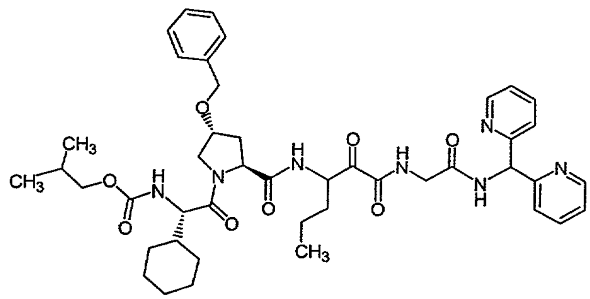


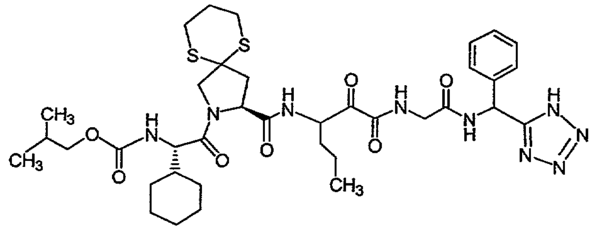


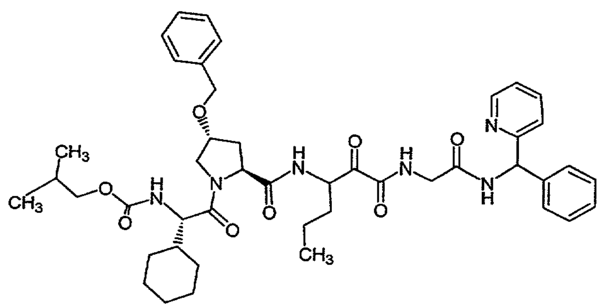


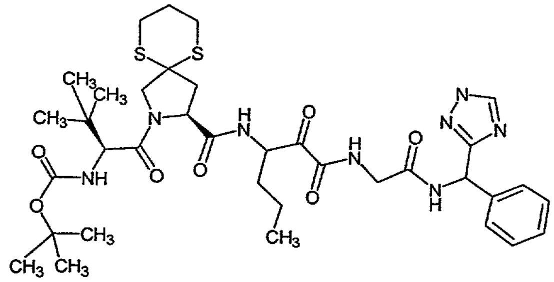


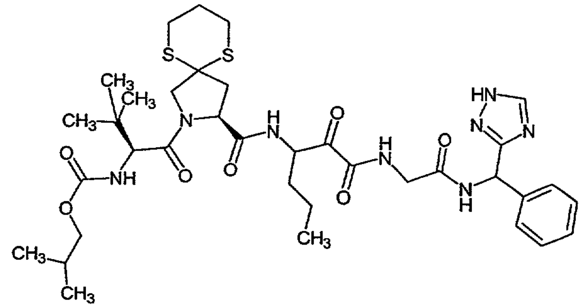


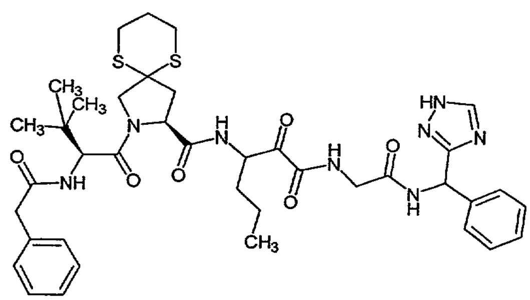


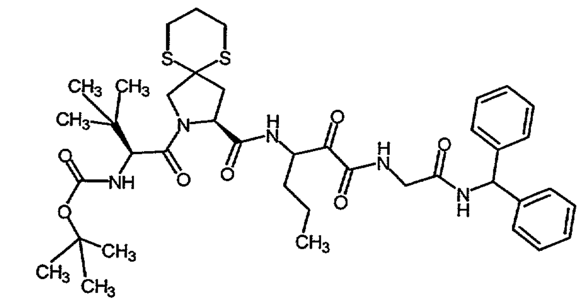


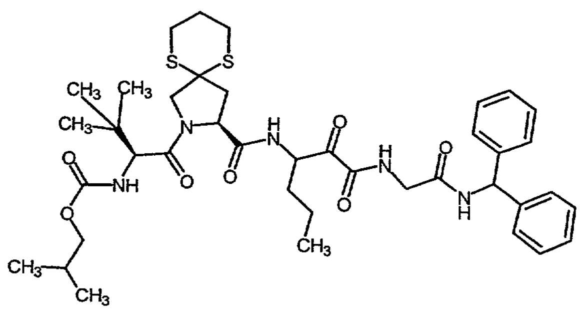


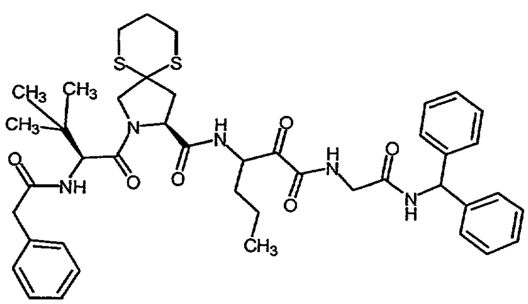


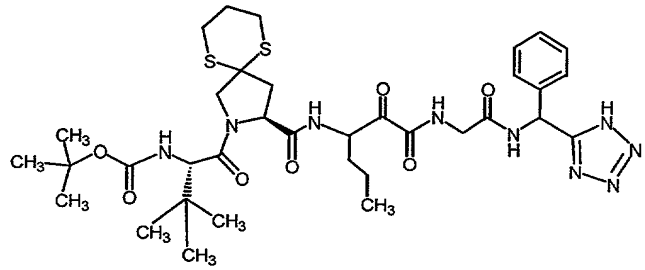


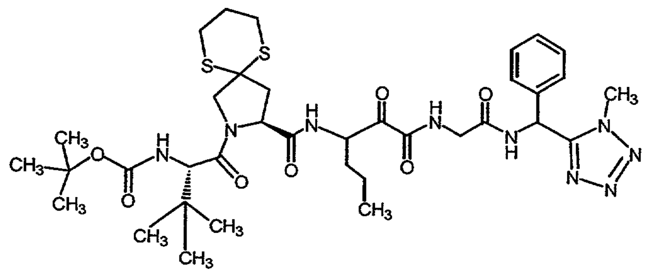


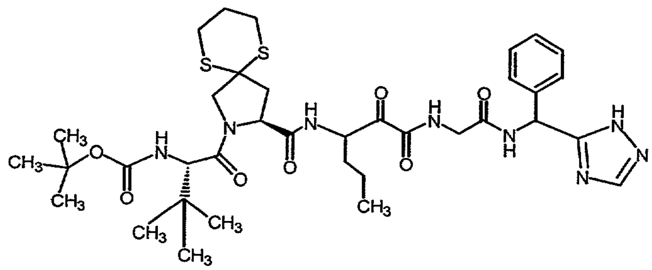


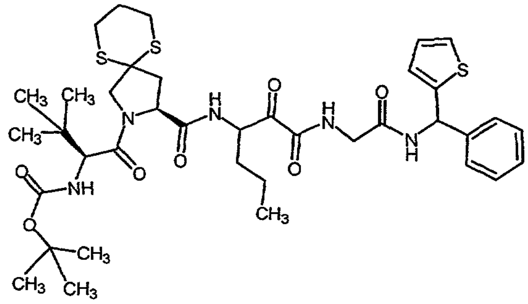


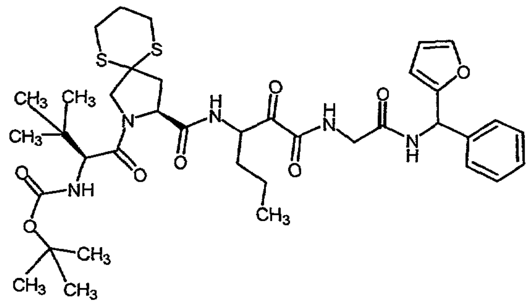


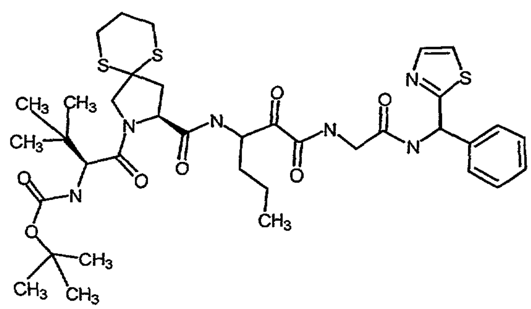


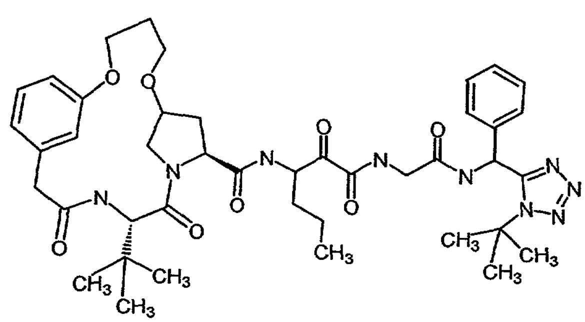






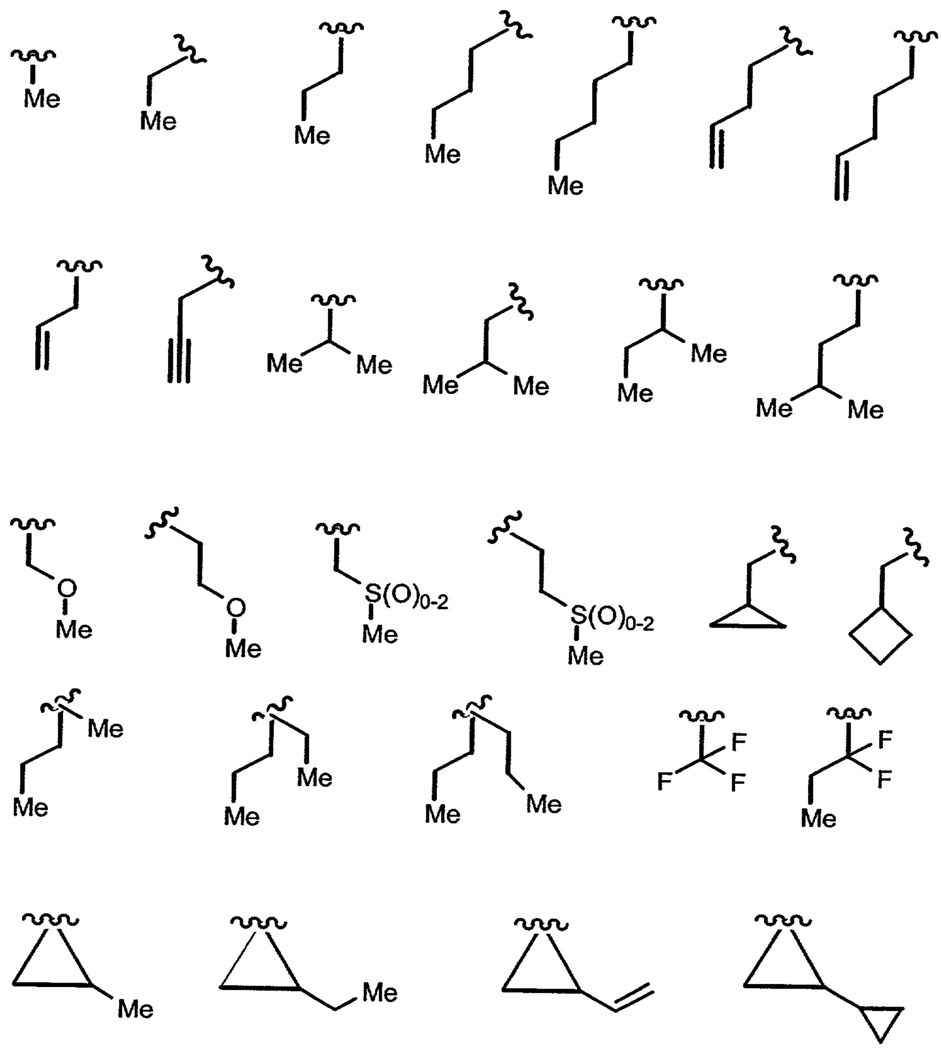




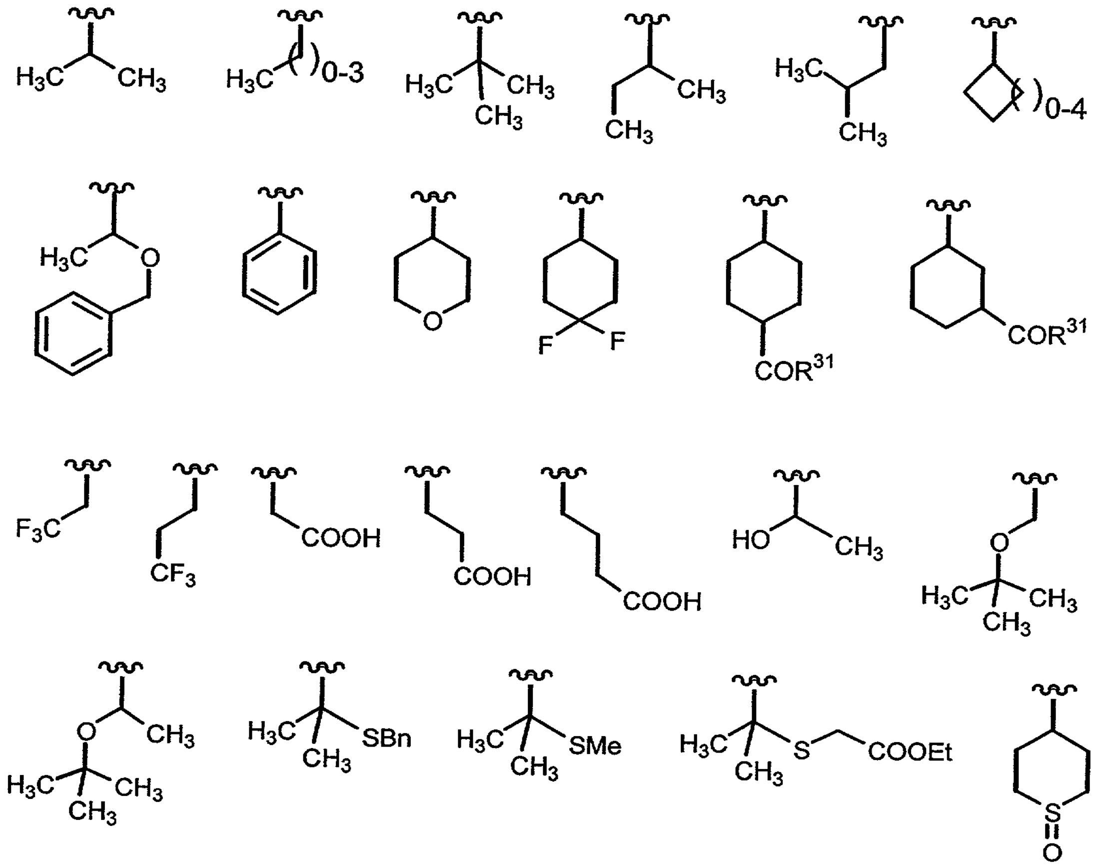


The following description of suitable moieties is applicable for compounds of Formulas I, II and III:

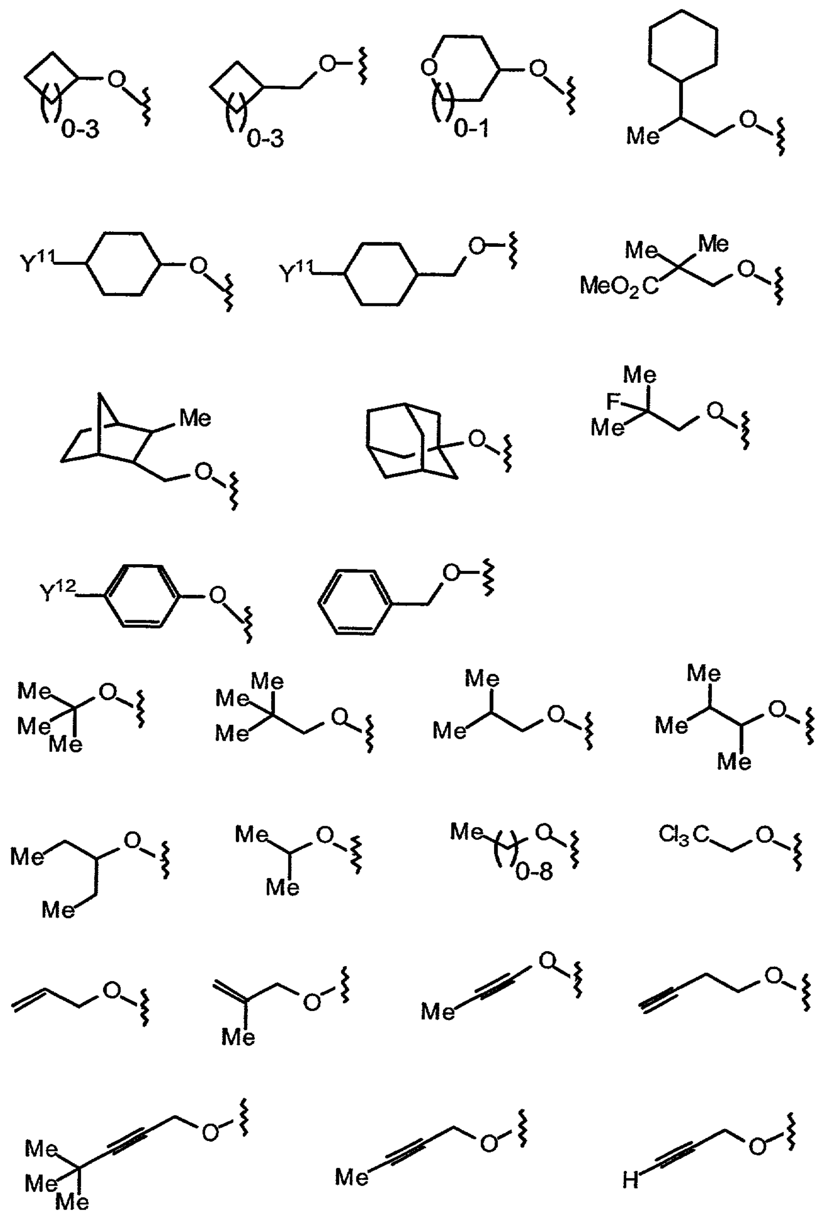
The following moieties are suitable P^1 moieties:



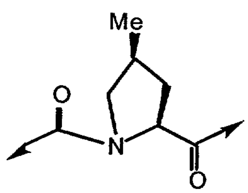
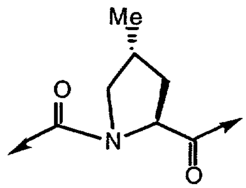
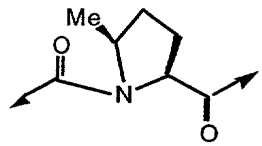
Also, the following moieties are suitable P^3 moieties:

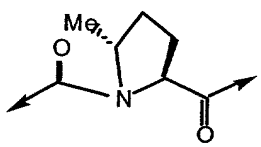
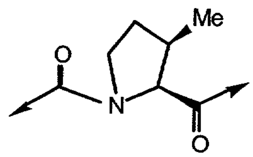
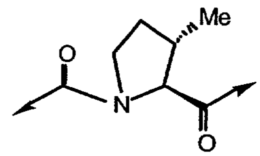


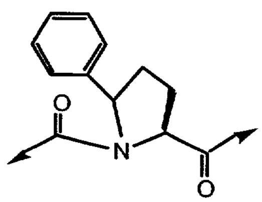
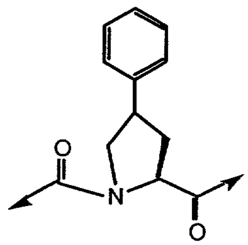
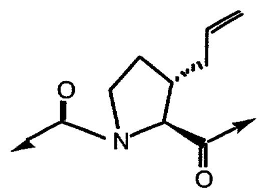
The following moieties are suitable **Y** moieties:

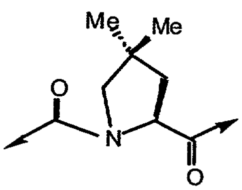
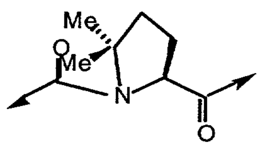
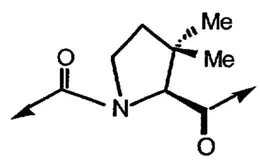


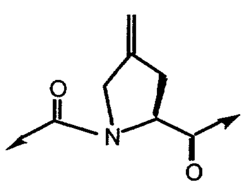
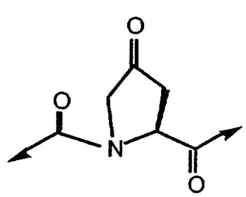
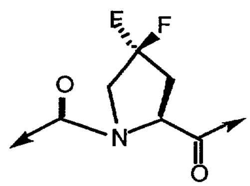
The following moieties are suitable **V-P2** moieties:

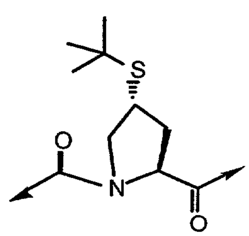
  



Depending upon their structure, the compounds of the invention may form pharmaceutically acceptable salts with organic or inorganic acids, or organic or inorganic bases. Examples of suitable acids for such salt formation are hydrochloric, sulfuric, phosphoric, acetic, citric, malonic,

salicylic, malic, fumaric, succinic, ascorbic, maleic, methanesulfonic and other mineral and carboxylic acids well known to those skilled in the art. For formation of salts with bases, suitable bases are, for example, NaOH, KOH, NH_4OH, tetraalkylammonium hydroxide, and the like.

In another embodiment, this invention provides pharmaceutical compositions comprising the inventive peptides as an active ingredient. The pharmaceutical compositions generally additionally comprise a pharmaceutically acceptable carrier diluent, excipient or carrier (described below and collectively referred to herein as carrier materials). Because of their HCV inhibitory activity, such pharmaceutical compositions possess utility in treating hepatitis C and related disorders.

In yet another embodiment, the present invention discloses methods for preparing pharmaceutical compositions comprising the inventive compounds as an active ingredient. In the pharmaceutical compositions and methods of the present invention, the active ingredients will typically be administered in admixture with suitable carrier materials suitably selected with respect to the intended form of administration, i.e. oral tablets, capsules (either solid-filled, semi-solid filled or liquid filled), powders for constitution, oral gels, elixirs, dispersible granules, syrups, suspensions, and the like, and consistent with conventional pharmaceutical practices. For example, for oral administration in the form of tablets or capsules, the active drug component may be combined with any oral non-toxic pharmaceutically acceptable inert carrier, such as lactose, starch, sucrose, cellulose, magnesium stearate, dicalcium phosphate, calcium sulfate, talc, mannitol, ethyl alcohol (liquid forms) and the like. Moreover, when desired or needed, suitable binders, lubricants, disintegrating agents and coloring agents may also be incorporated in the mixture. Powders and tablets may be comprised of from about 5 to about 95 percent inventive composition. Suitable binders include starch, gelatin, natural sugars, corn sweeteners, natural and synthetic gums such as

acacia, sodium alginate, carboxymethylcellulose, polyethylene glycol and waxes. Among the lubricants there may be mentioned for use in these dosage forms, boric acid, sodium benzoate, sodium acetate, sodium chloride, and the like. Disintegrants include starch, methylcellulose, guar gum and the like. Sweetening and flavoring agents and preservatives may also be included where appropriate. Some of the terms noted above, namely disintegrants, diluents, lubricants, binders and the like, are discussed in more detail below.

Additionally, the compositions of the present invention may be formulated in sustained release form to provide the rate controlled release of any one or more of the components or active ingredients to optimize the therapeutic effects, i.e. HCV inhibitory activity and the like. Suitable dosage forms for sustained release include layered tablets containing layers of varying disintegration rates or controlled release polymeric matrices impregnated with the active components and shaped in tablet form or capsules containing such impregnated or encapsulated porous polymeric matrices.

Liquid form preparations include solutions, suspensions and emulsions. As an example may be mentioned water or water-propylene glycol solutions for parenteral injections or addition of sweeteners and pacifiers for oral solutions, suspensions and emulsions. Liquid form preparations may also include solutions for intranasal administration.

Aerosol preparations suitable for inhalation may include solutions and solids in powder form, which may be in combination with a pharmaceutically acceptable carrier such as inert compressed gas, e.g. nitrogen.

For preparing suppositories, a low melting wax such as a mixture of fatty acid glycerides such as cocoa butter is first melted, and the active ingredient is dispersed homogeneously therein by stirring or similar mixing.

The molten homogeneous mixture is then poured into convenient sized molds, allowed to cool and thereby solidify.

Also included are solid form preparations which are intended to be converted, shortly before use, to liquid form preparations for either oral or parenteral administration. Such liquid forms include solutions, suspensions and emulsions.

The compounds of the invention may also be deliverable transdermally. The transdermal compositions may take the form of creams, lotions, aerosols and/or emulsions and can be included in a transdermal patch of the matrix or reservoir type as are conventional in the art for this purpose.

Preferably the compound is administered orally.

Preferably, the pharmaceutical preparation is in a unit dosage form. In such form, the preparation is subdivided into suitably sized unit doses containing appropriate quantities of the active components, e.g., an effective amount to achieve the desired purpose.

The quantity of the inventive active composition in a unit dose of preparation may be generally varied or adjusted from about 1.0 milligram to about 1,000 milligrams, preferably from about 1.0 to about 950 milligrams, more preferably from about 1.0 to about 500 milligrams, and typically from about 1 to about 250 milligrams, according to the particular application. The actual dosage employed may be varied depending upon the patient's age, sex, weight and severity of the condition being treated. Such techniques are well known to those skilled in the art.

Generally, the human oral dosage form containing the active ingredients can be administered 1 or 2 times per day. The amount and frequency of the administration will be regulated according to the judgment of the attending clinician. A generally recommended daily dosage regimen for oral administration may range from about 1.0 milligram to about 1,000 milligrams per day, in single or divided doses.

Some useful terms are described below:

Capsule - refers to a special container or enclosure made of methyl cellulose, polyvinyl alcohols, or denatured gelatins or starch for holding or containing compositions comprising the active ingredients. Hard shell capsules are typically made of blends of relatively high gel strength bone and pork skin gelatins. The capsule itself may contain small amounts of dyes, opaquing agents, plasticizers and preservatives.

Tablet- refers to a compressed or molded solid dosage form containing the active ingredients with suitable diluents. The tablet can be prepared by compression of mixtures or granulations obtained by wet granulation, dry granulation or by compaction.

Oral gel- refers to the active ingredients dispersed or solubilized in a hydrophillic semi-solid matrix.

Powder for constitution refers to powder blends containing the active ingredients and suitable diluents which can be suspended in water or juices.

Diluent - refers to substances that usually make up the major portion of the composition or dosage form. Suitable diluents include sugars such as lactose, sucrose, mannitol and sorbitol; starches derived from wheat, corn, rice and potato; and celluloses such as microcrystalline cellulose. The amount of diluent in the composition can range from about 10 to about 90% by weight of the total composition, preferably from about 25 to about 75%, more preferably from about 30 to about 60% by weight, even more preferably from about 12 to about 60%.

Disintegrant - refers to materials added to the composition to help it break apart (disintegrate) and release the medicaments. Suitable disintegrants include starches; "cold water soluble" modified starches such as sodium carboxymethyl starch; natural and synthetic gums such as locust bean, karaya, guar, tragacanth and agar; cellulose derivatives such as methylcellulose and sodium carboxymethylcellulose; microcrystalline

celluloses and cross-linked microcrystalline celluloses such as sodium croscarmellose; alginates such as alginic acid and sodium alginate; clays such as bentonites; and effervescent mixtures. The amount of disintegrant in the composition can range from about 2 to about 15% by weight of the composition, more preferably from about 4 to about 10% by weight.

Binder - refers to substances that bind or "glue" powders together and make them cohesive by forming granules, thus serving as the "adhesive" in the formulation. Binders add cohesive strength already available in the diluent or bulking agent. Suitable binders include sugars such as sucrose; starches derived from wheat, corn rice and potato; natural gums such as acacia, gelatin and tragacanth; derivatives of seaweed such as alginic acid, sodium alginate and ammonium calcium alginate; cellulosic materials such as methylcellulose and sodium carboxymethylcellulose and hydroxypropylmethylcellulose; polyvinylpyrrolidone; and inorganics such as magnesium aluminum silicate. The amount of binder in the composition can range from about 2 to about 20% by weight of the composition, more preferably from about 3 to about 10% by weight, even more preferably from about 3 to about 6% by weight.

Lubricant - refers to a substance added to the dosage form to enable the tablet, granules, etc. after it has been compressed, to release from the mold or die by reducing friction or wear. Suitable lubricants include metallic stearates such as magnesium stearate, calcium stearate or potassium stearate; stearic acid; high melting point waxes; and water soluble lubricants such as sodium chloride, sodium benzoate, sodium acetate, sodium oleate, polyethylene glycols and d'l-leucine. Lubricants are usually added at the very last step before compression, since they must be present on the surfaces of the granules and in between them and the parts of the tablet press. The amount of lubricant in the composition can range from about 0.2 to about 5% by weight of the composition,

preferably from about 0.5 to about 2%, more preferably from about 0.3 to about 1.5% by weight.

Glident - material that prevents caking and improve the flow characteristics of granulations, so that flow is smooth and uniform. Suitable glidents include silicon dioxide and talc. The amount of glident in the composition can range from about 0.1% to about 5% by weight of the total composition, preferably from about 0.5 to about 2% by weight.

Coloring agents - excipients that provide coloration to the composition or the dosage form. Such excipients can include food grade dyes and food grade dyes adsorbed onto a suitable adsorbent such as clay or aluminum oxide. The amount of the coloring agent can vary from about 0.1 to about 5% by weight of the composition, preferably from about 0.1 to about 1%.

Bioavailability - refers to the rate and extent to which the active drug ingredient or therapeutic moiety is absorbed into the systemic circulation from an administered dosage form as compared to a standard or control.

Conventional methods for preparing tablets are known. Such methods include dry methods such as direct compression and compression of granulation produced by compaction, or wet methods or other special procedures. Conventional methods for making other forms for administration such as, for example, capsules, suppositories and the like are also well known.

Another embodiment of the invention discloses the use of the pharmaceutical compositions disclosed above for treatment of diseases such as, for example, hepatitis C and the like. The method comprises administering a therapeutically effective amount of the inventive pharmaceutical composition to a patient having such a disease or diseases and in need of such a treatment.

As stated earlier, the invention includes tautomers, rotamers, enantiomers and other stereoisomers of the compounds also. Thus, as

one skilled in the art appreciates, some of the inventive compounds may exist in suitable isomeric forms. Such variations are contemplated to be within the scope of the invention.

Another embodiment of the invention discloses a method of making the compounds disclosed herein. The compounds may be prepared by several techniques known in the art. Representative illustrative procedures are outlined in the following reaction schemes. It is to be understood that while the following illustrative schemes describe the preparation of a few representative inventive compounds, suitable substitution of any of both the natural and unnatural amino acids will result in the formation of the desired compounds based on such substitution. Such variations are contemplated to be within the scope of the invention.

Abbreviations which are used in the descriptions of the schemes, preparations and the examples that follow are:

THF: Tetrahydrofuran
DMF: *N*,*N*-Dimethylformamide
EtOAc: Ethyl acetate
AcOH: Acetic acid
HOOBt: 3-Hydroxy-1,2,3-benzotriazin-4(3*H*)-one
EDCI: 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
NMM: *N*-Methylmorpholine
ADDP: 1,1'-(Azodicarboxyl)dipiperidine
DEAD: Diethylazodicarboxylate
MeOH: Methanol
EtOH: Ethanol
Et_2O: Diethyl ether
PyBrOP: Bromo-*tris*-pyrrolidinophosphonium hexafluorophosphate
Bn: Bzl:Benzyl
Boc: tert-Butyloxycarbonyl

Cbz: Benzyloxycarbonyl

Ts: p-toluenesulfonyl

Me: Methyl

Bs: p-bromobenzenesulfonyl

DCC: dicyclohexylcarbodiimide

DMSO: dimethylsulfoxide

SEM: (trimethylsiyl)ethoxymethyl

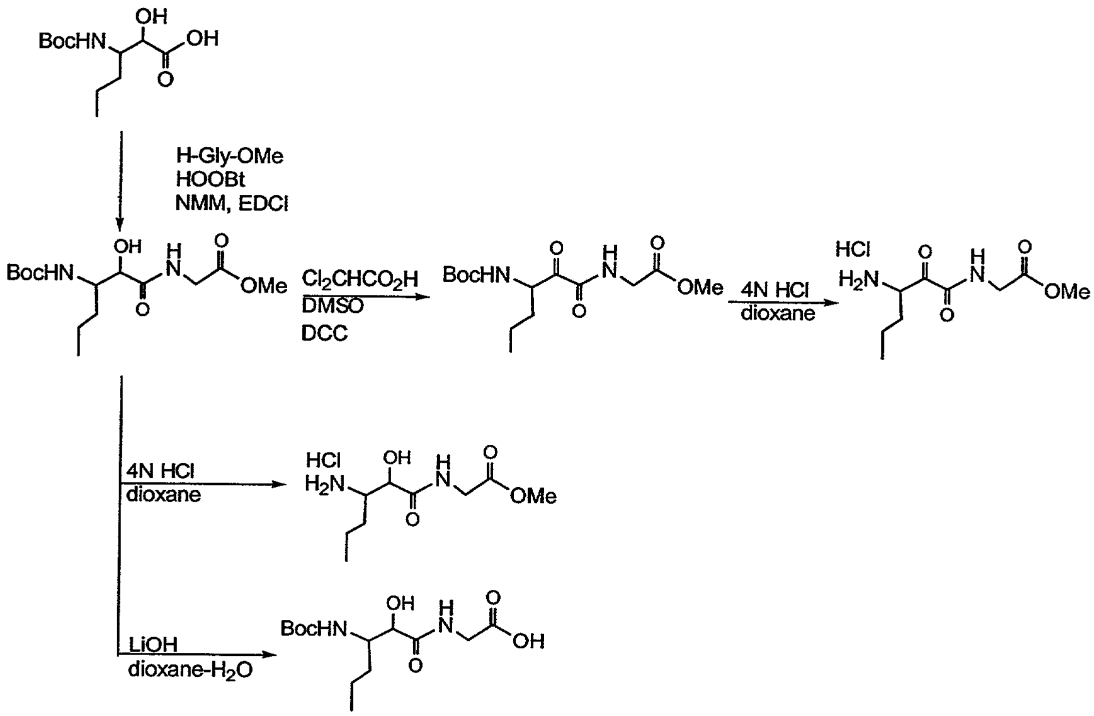
TEMPO: 2,2,6,6-tetramethyl-1-piperidinyloxy free radical

HATU: O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium

General Preparative Schemes:

The following schemes describe generally methods of synthesis of the intermediates and the inventive diaryl peptides of the present invention.

SCHEME 1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OH
OH
O
H-Gly-OMe
HOOBt
NMM, EDCI
BocHN
OH
H
N
O
OMe
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DMSO
DCC
BocHN
O
H
N
O
OMe
4N HCl
dioxane
HCl
H_2N
O
H
N
O
OMe
4N HCl
dioxane
HCl
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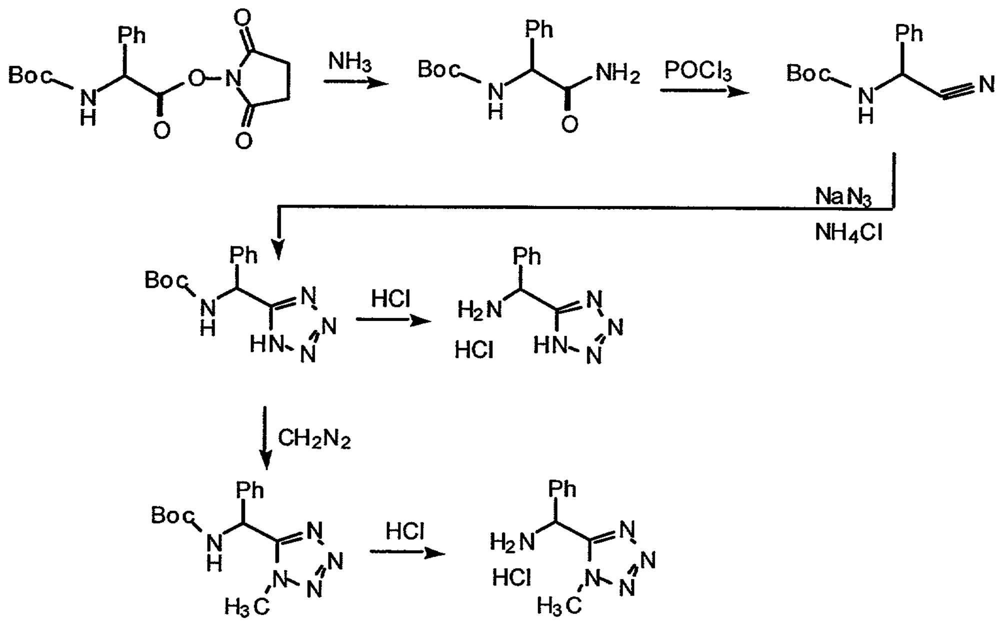
OH

SCHEME 2



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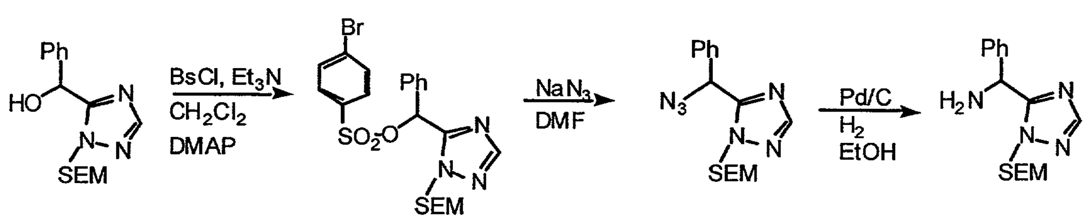

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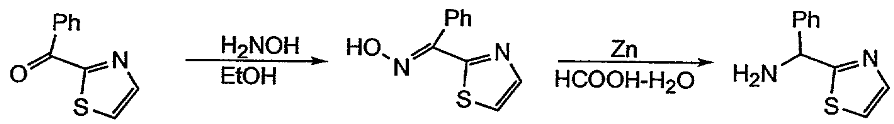

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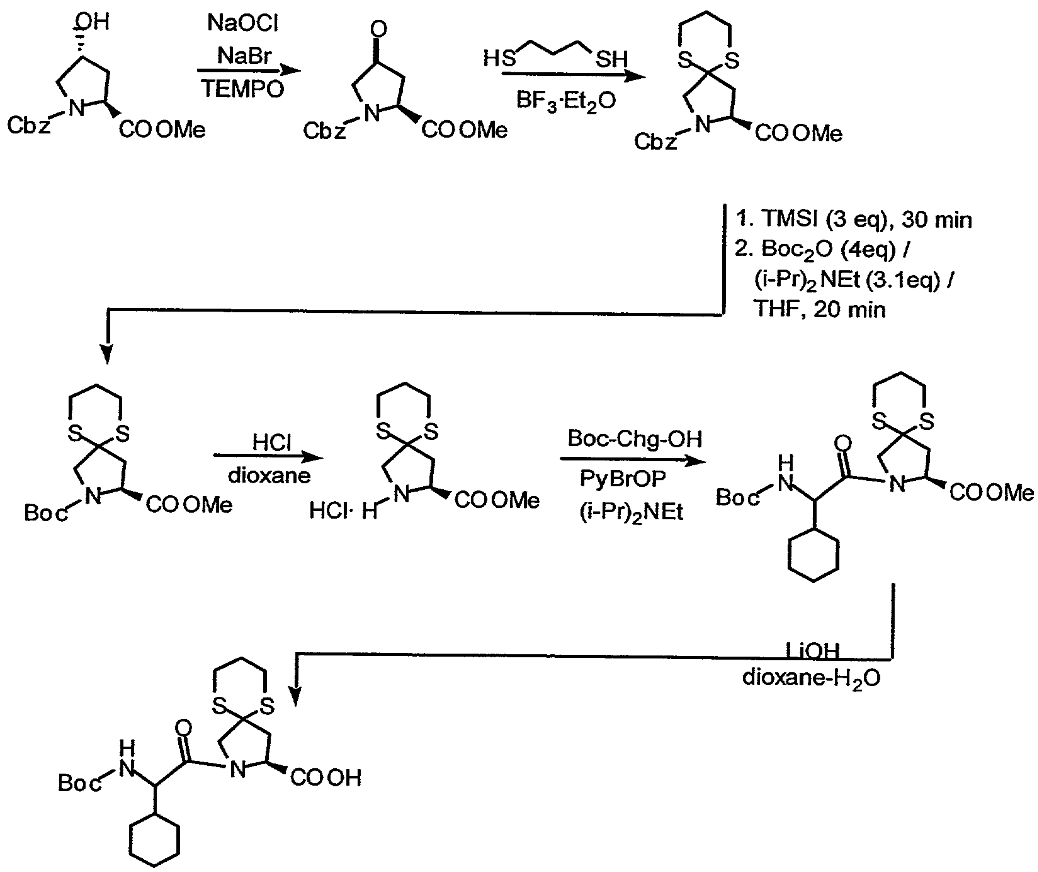
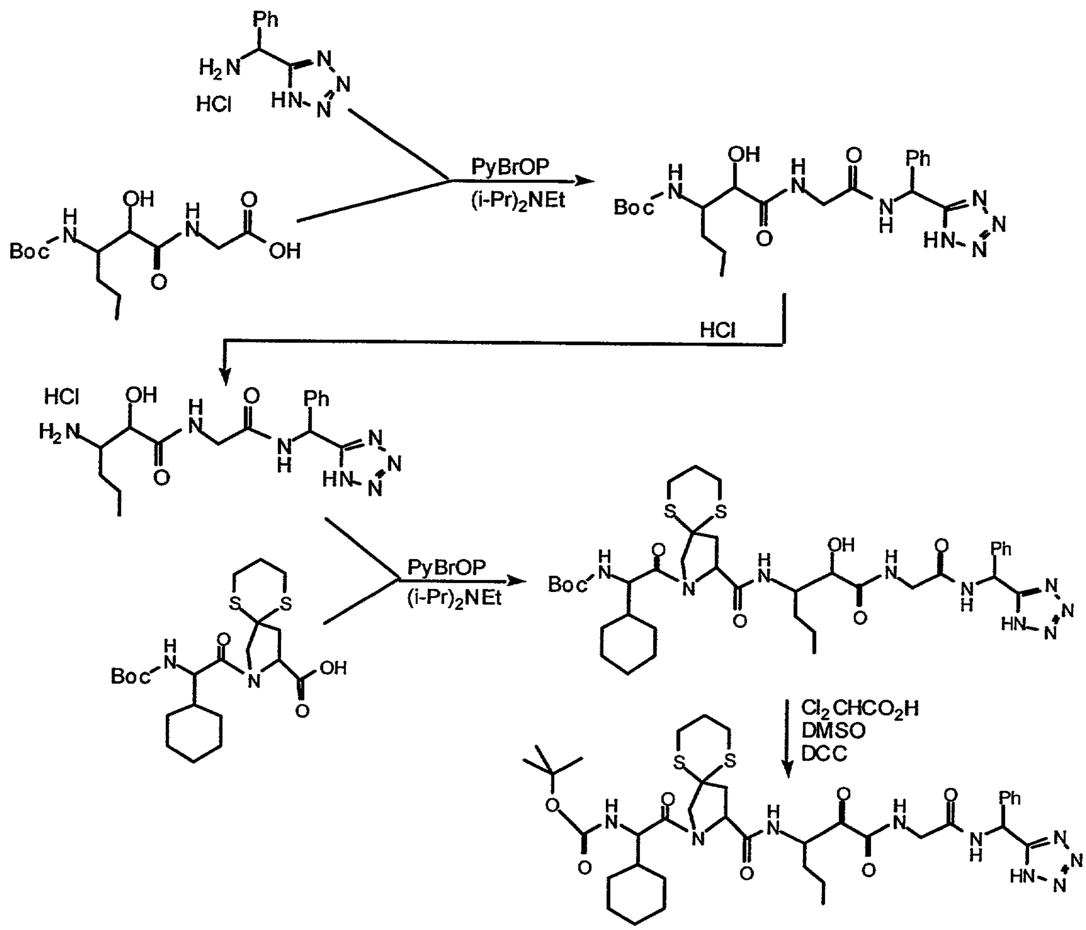
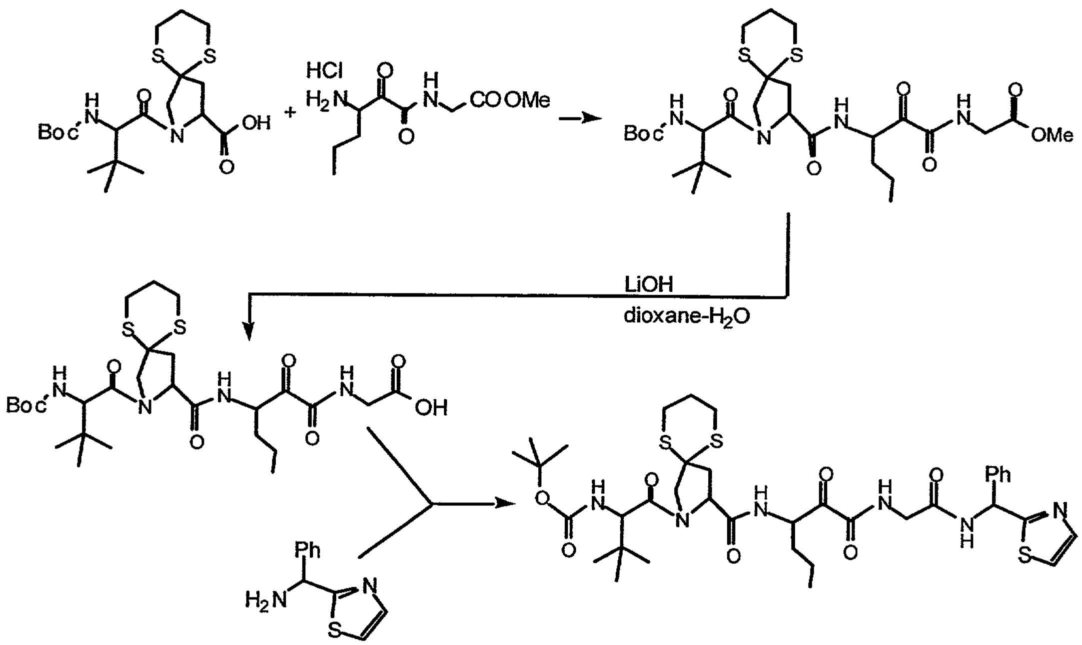


SCHEME 6

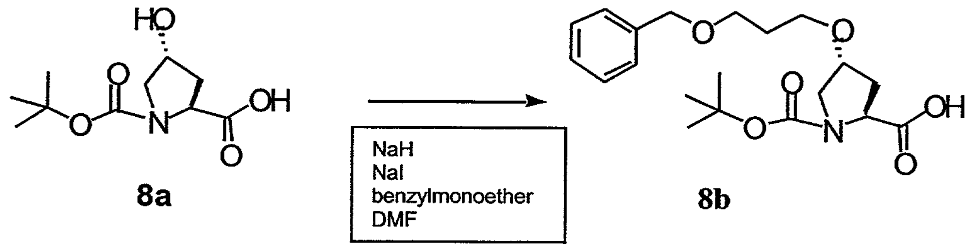


SCHEME 7

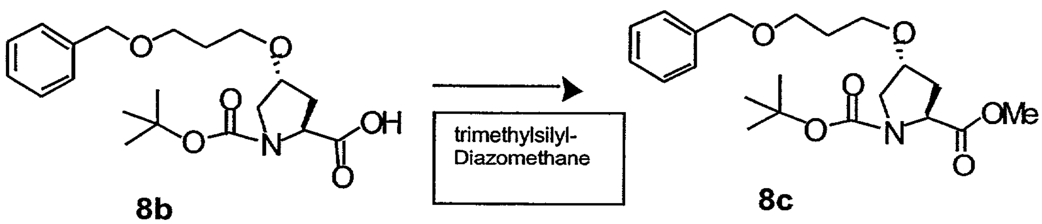


SCHEME 8

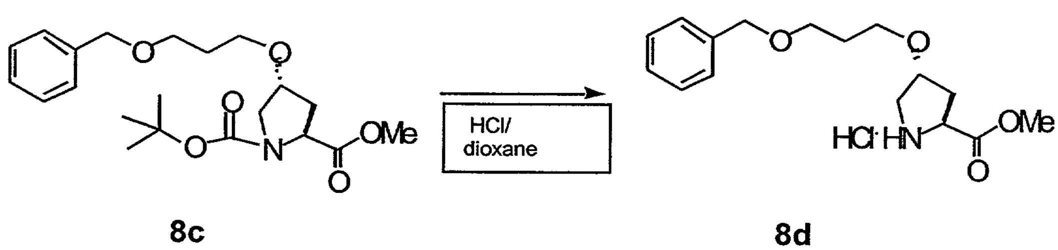
Step A



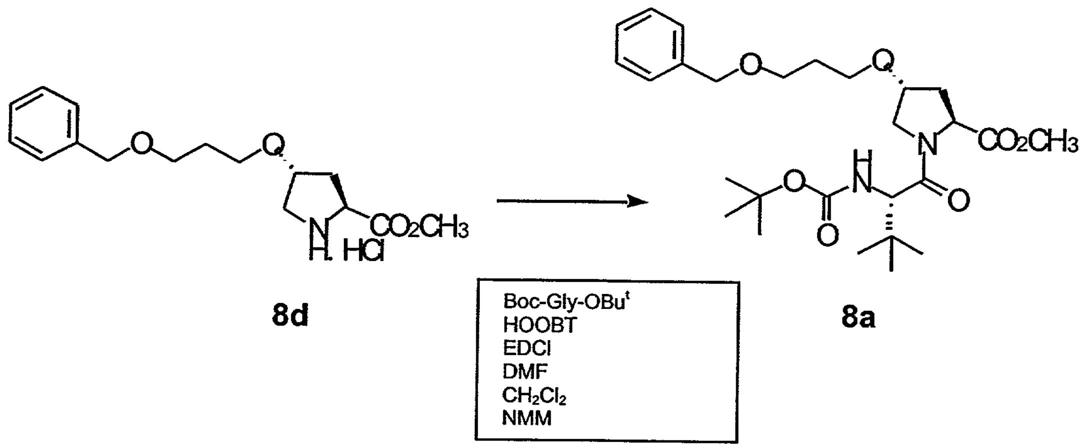
Step B



Step C

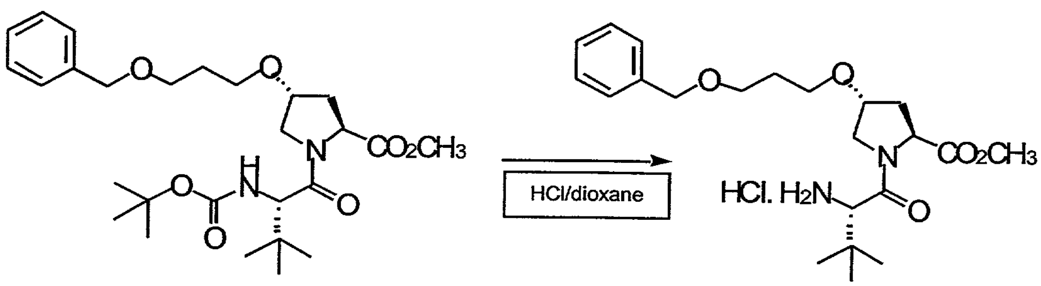


Step D



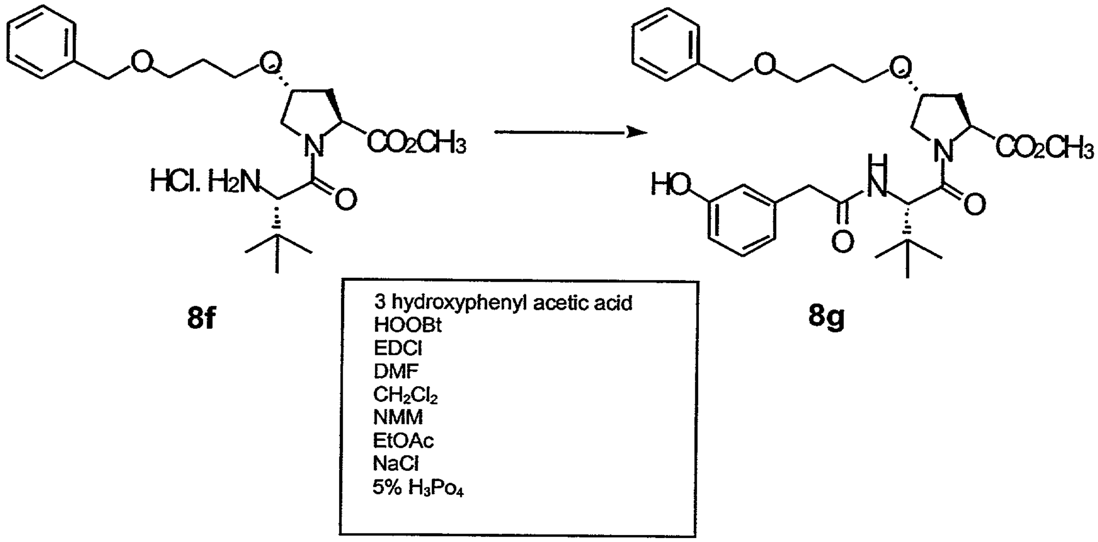
8d **8a**

Step E



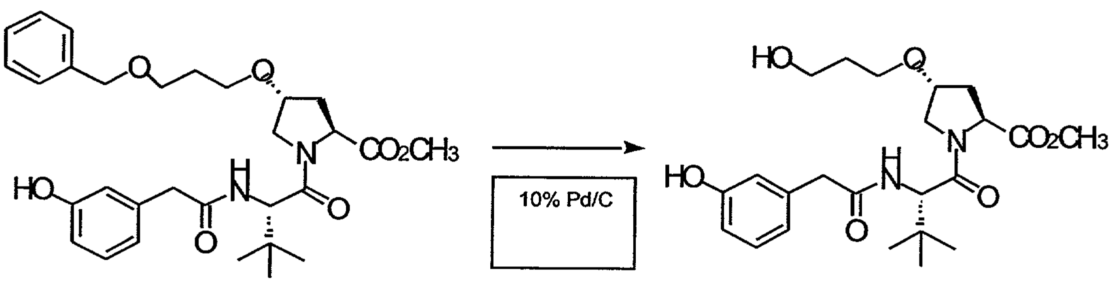
8e **8f**

Step F



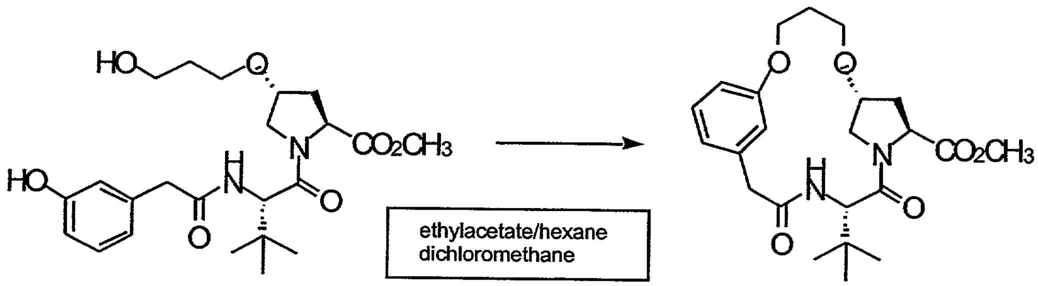
8f **8g**

Step G



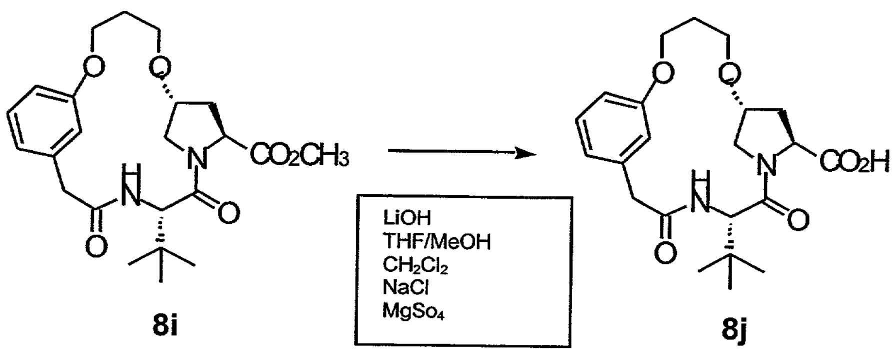
8g **8h**

Step H



8h 8i

Step I

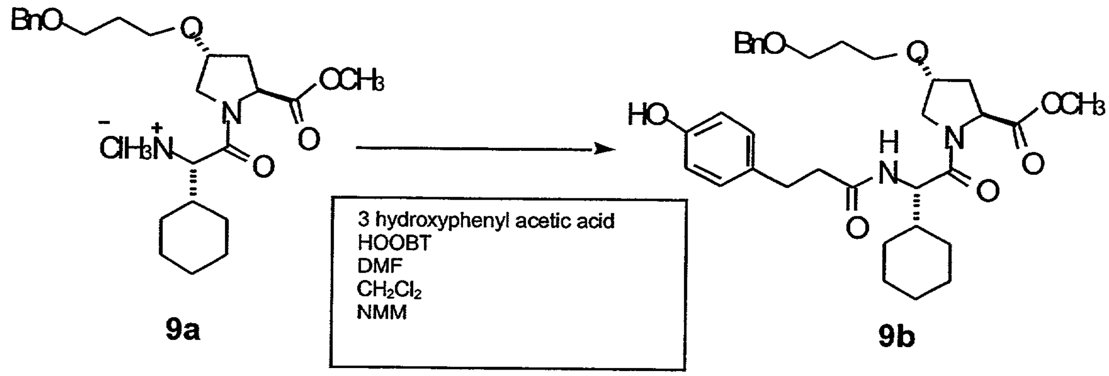


8i 8j

SCHEME 9

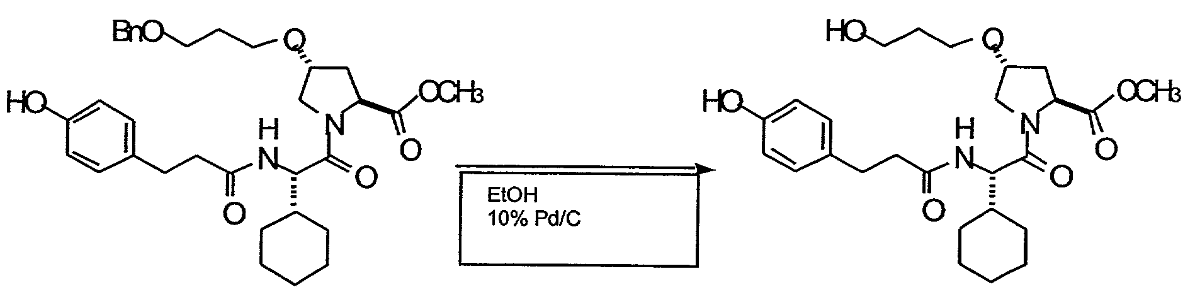
Compound **9a** was prepared analogous to Scheme 8 Steps A to F.

Step A



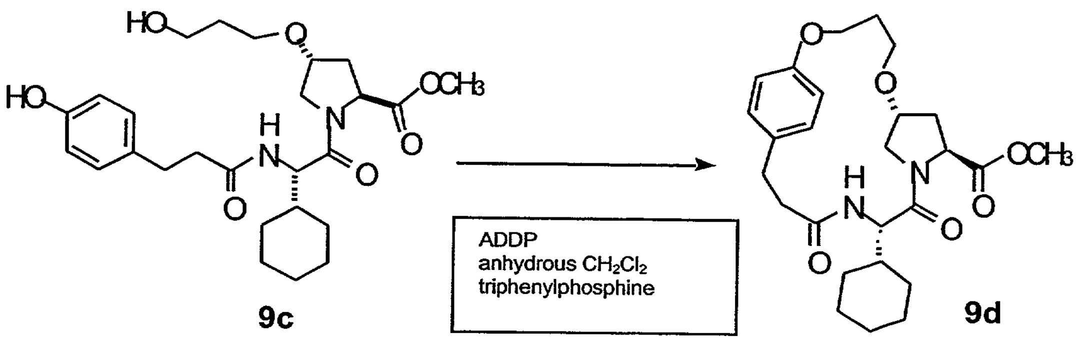
9a **9b**

Step B



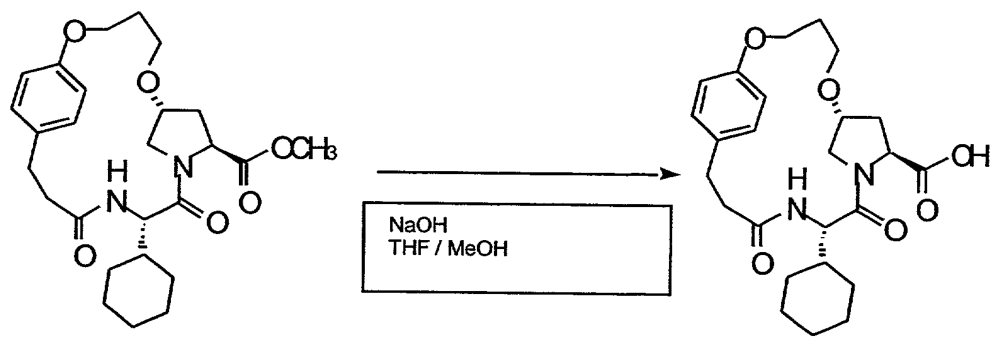
9b **9c**

Step C



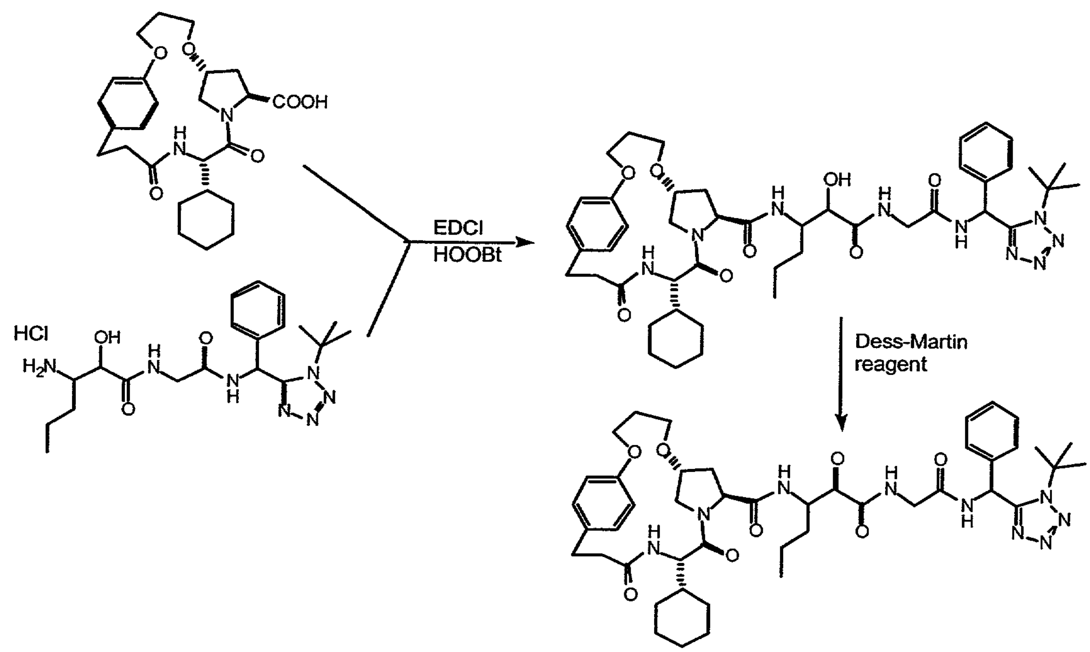
9c 9d

Step D



9d 9e

SCHEME 10



Preparation of Intermediates:

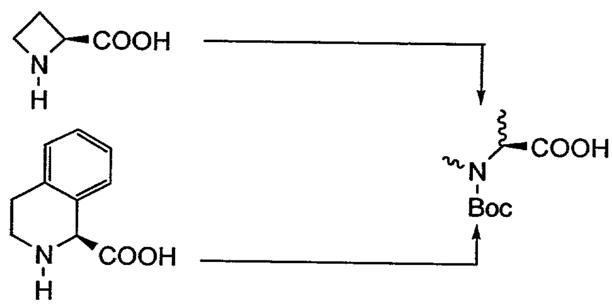
The procedures to modify an amino acid with N-Boc, N-Cbz, COOBzl, COOBu^t, OBzl, OBu^t, COOMe, both putting them on or taking them off in the presence of each other in various combinations, are generally well known to those skilled in the art. Any modifications from the known procedures are noted herein.

Commercially available intermediates:

The following amino acids, used as amino acid units in the preparation of the various inventive compounds, are commercially available, and were converted to their N-Boc derivatives with di-*tert*-butyldicarbonate, using known procedures.

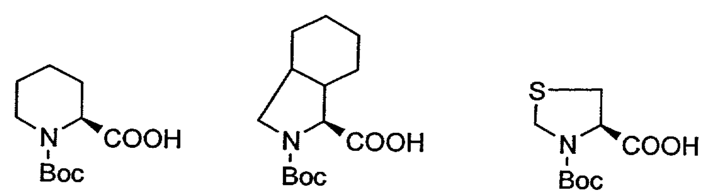
Aldrich Chemical Co.

Tyger Scientific Inc.,
Monmouth Junction, New Jersey

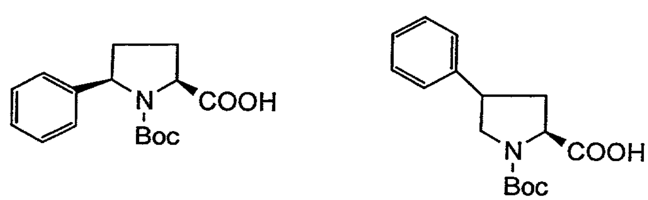


The following N-Boc-amino acids, used as P2 units, are commercially available.

Bachem Biosciences, Inc.

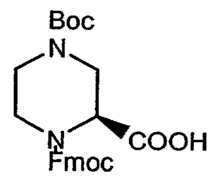


Neosystems,
Princeton, New Jersey



The following N-Boc-amino acid, used as P2 unit, is commercially available. After coupling the carboxylic acid, the Fmoc is removed by known treatment with piperidine before subsequent coupling.

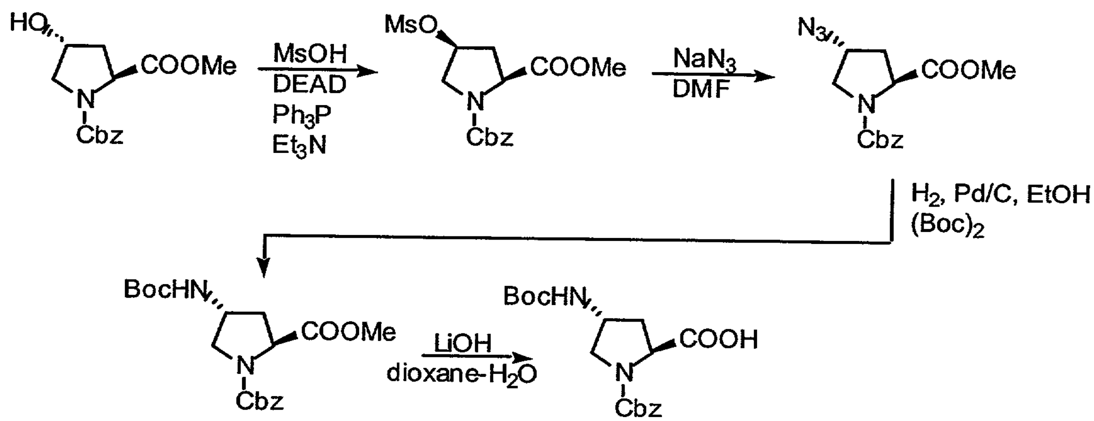
RSP Amino Acid Analogues, Inc.
Worchester, Massachusetts



Example A

Certain intermediates which were not commercially available were synthesized, as needed, by following the procedures given below:

I.



II. Mesylate:

A mixture of triphenylphosphine (8.7 g), toluene (200 mL), and methanesulfonic acid (2.07 mL) was stirred at 15°C while slowly adding diethylazidodicarboxylate (7.18 g) to maintain the temperature below 35°C. The mixture was cooled to 20°C, and the N-Boc amino acid (7.4 g, Bachem Biosciences, Inc.), and Et_3N (1.45 mL) were added, and then the mixture was stirred at 70°C for 5 hr. The mixture was cooled to 5°C, the organic supernatant decanted, and solvent was removed from it *in vacuo*. The residue was stirred with Et_2O (200 mL) until a precipitate deposits, the mixture was filtered, and the ethereal solution was chromatographed on silica gel (5:95 to 20:80 EtOAc-Et_2O) to obtain the product (9.3 g), which was carried into the next step.

III. Azide

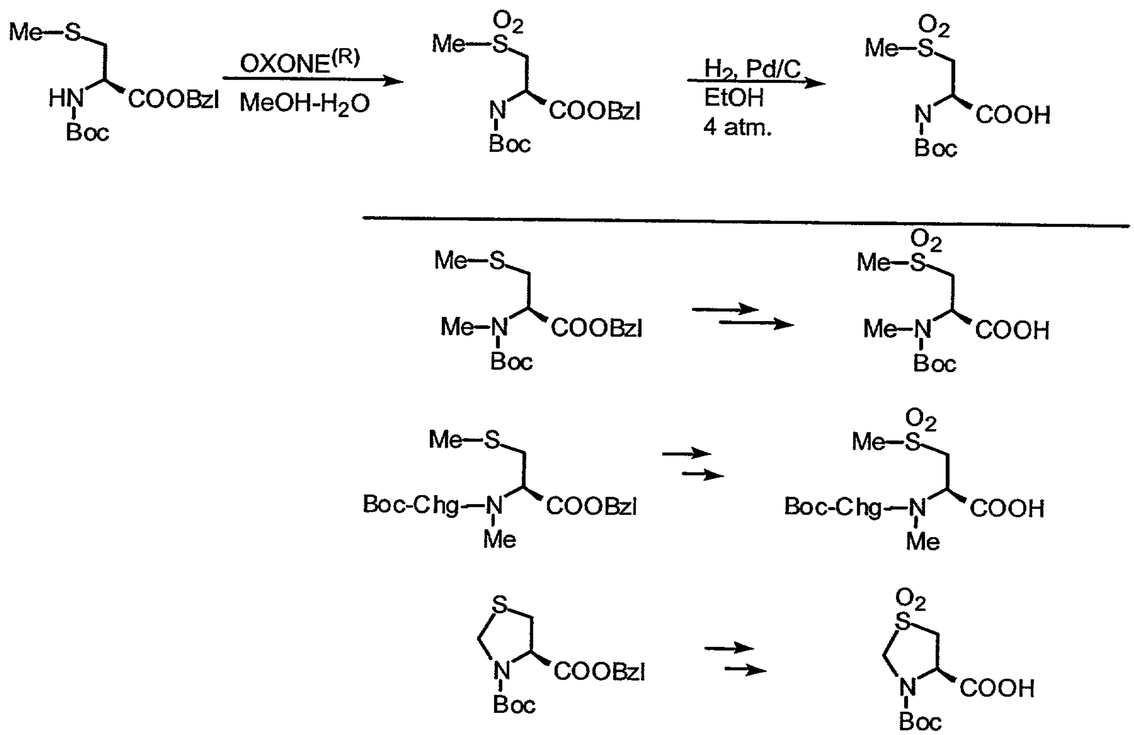
Sodium azide (1.98 g) was added to a solution of the product of the step above (9.3 g) in DMF (100 mL), and the mixture stirred at 70 °C for 8 hr. The mixture was cooled, and poured into 5% aqueous $NaHCO_3$, and extracted with EtOAc. The organic layer was washed with brine, then dried over anhydrous $MgSO_4$. The mixture was filtered, and the filtrate evaporated *in vacuo*, to obtain the product (6.2 g), which was carried into the next step.

IV. N-Cbz(4-N-Boc)-OMe

A solution of the product of the step above (0.6 g) in dioxane (40 mL) was treated with di-*tert*-butyldicarbonate (0.8 g), 10% Pd-C (0.03g), and hydrogen at one atmosphere for 18 hr. The mixture was filtered, the filtrate evaporated *in vacuo*, and the residue chromatographed on silica gel (1:1 to 2:1 Et_2O-hexane) to obtain the product.

V. N-Cbz(4-N-Boc)-OH was prepared using known ester hydrolysis using LiOH.

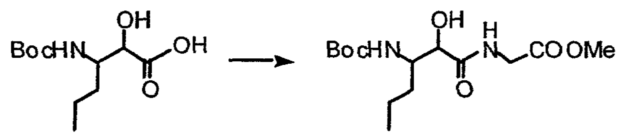
VI. Sulfones by Oxidation:



These were prepared by following the procedure of U. Larsson, *et al.*, *Acta Chem. Scan.*, (1994), <u>48(6)</u>, 517-525. A solution of oxone(R) (20.2 g, from Aldrich Chemical Co.) in water (110 mL) was added slowly to a 0°C solution of the sulfide (7.2 g, from Bachem Biosciences, Inc.) in MeOH (100 mL). The cold bath was removed and the mixture stirred for 4 hr. The mixture was concentrated to 1/2 volume on a rotary evaporator, cold water (100 mL) added, extracted with EtOAc, the extract washed with brine, and then it was dried over anhydrous $MgSO_4$. The mixture was filtered, and the filtrate evaporated *in vacuo*, to obtain the product as a white solid (7.7 g). A portion was crystallized from $(i\text{-Pr})_2O$ to obtain an analytical sample, $[\alpha]_D$ +8.6 (c 0.8, $CHCl_3$). Using the same procedure, the other sulfides shown were oxidized to sulfones to lead to the subject targets.

Example 1

Step A.

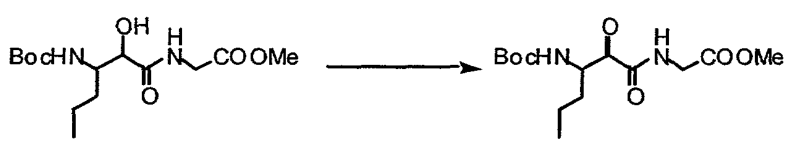


1A

To a stirred solution of compound (4.01) (12 g) prepared according to S. L. Harbeson *et al.*, *J.Med.Chem.* 37 (18), 2918-2929 (1994), in CH_2Cl_2 (150 mL) at -20°C was added HOOBt (7.5 g), *N*-methyl morpholine (6.0 mL) and EDCI (10 g). The reaction mixture was stirred for 10 minutes, followed by the addition of HCl·H_2N-Gly-OMe (6.8 g). The resulting solution was stirred at -20°C for 2 hrs, then kept at 8°C overnight. The solution was concentrated to dryness, then diluted with EtOAc (150 mL). The EtOAc solution was then washed twice with saturated $NaHCO_3$, H_2O, 5% H_3PO_4, and brine, dried over Na_2SO_4, filtered and concentrated to dryness to give the product, $C_{14}H_{26}N_2O_6$ (318.37) LRMS *m/z* MH^+= 319.3.

Example 1

Step B

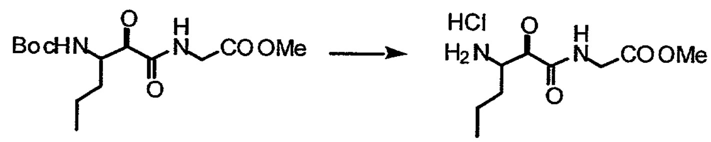


1B

A mixture of the product from Step A above (5.7 g), dichloromethane (200 mL), methyl sulfoxide (12 mL), and 2,2-dichloroacetic acid (3.2 mL) was stirred at 5°C. To this was added a solution of 1 M dicyclohexylcarbodiimide in CH_2Cl_2 (23 mL), and the

resulting mixture was stirred cold for 5 min., at room temperature for 3 h. A solution of oxalic acid (0.6 g) in methanol (6 mL) was added to destroy excess oxidant, stirred for 15 min., and then filtered to remove the precipitated urea. The filtrate was concentrated *in vacuo*, the remainder diluted with excess ethyl acetate, and washed with cold 0.1 N NaOH, then cold 0.2 N H_3PO_4, then brine. The organic solution was dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. The residue was chromatographed on silica gel, eluting with a gradient of EtOAc-CH_2Cl_2 (5:95 to 1:1) to obtain the title compound as an oil which solidifies to a wax slowly on standing (5 g, 88% yield) $C_{14}H_{24}N_2O_6$ (316.35),

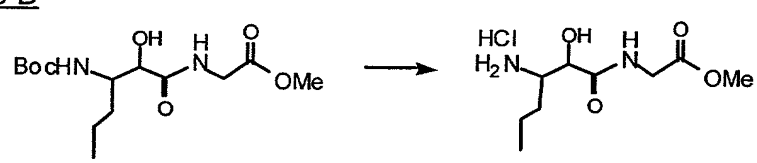
Example 1
Step C



1C

Treat the product of the previous step with a 4N solution of HCl in dioxane (Aldrich Chemical Co.) for 0.5 hr. concentrate the filtrate *in vacuo* in a 30°C water bath, and triturate the residue with Et_2O. Filter the mixture to leave the product compound as a white powder, $C_9H_{16}N_2O_4 \cdot HCl$ (252.70), which was used subsequently without further purification.

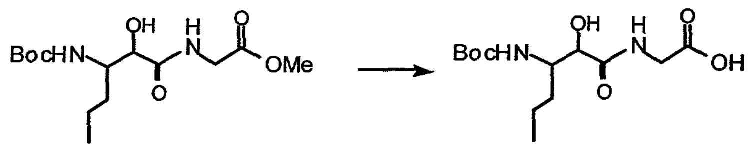
Example 1
Step D



Use the procedure of step C. above to treat the product of step A. above to obtain the product as a white powder, $C_9H_{18}N_2O_4 \cdot HCl$ (254.71).

Example 1

Step E

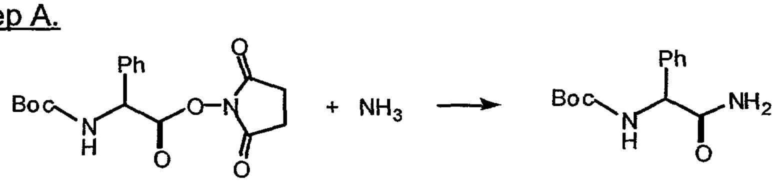


1E

Treat a solution of the product from Step A. above (8.3 g) in dioxane (150 mL) at 20°C with 1N aqueous LiOH (26 mL) and stir for 2 h. Pour the mixture into a solution of 10% aqueous KH_2PO_4 (500 mL), H_3PO_4 (2 mL), and saturated brine (300 mL); and then extract with EtOAc. Wash the extract with brine, dry it anhydrous $MgSO_4$, filter the mixture, and evaporate the filtrate *in vacuo* to leave the product as a white powder, $C_{13}H_{24}N_2O_6$ (304.34), LRMS (FAB) M+1 = 305.3.

Example 2

Step A.

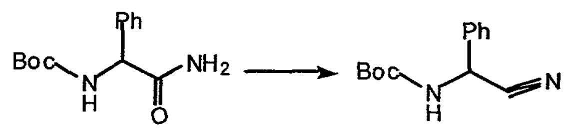


Treat a solution of N-Boc-phenylglycine N-hydoxysuccinimide ester (1.66 g; Bachem Biosciences, Inc.) in dichloromethane (CH_2Cl_2, 20 mL) with a solution of 0.5 M NH_3/dioxane (Aldrich Chemical Co.) (18.5 mL) at 5°C, then allow to warm and stir at room temperature for 4 hr. Suction-filter the mixture, add the filtrate to aq. 5% KH_2PO_4 (150 mL), then extract

with ethyl acetate (EtOAc, 200 mL). Wash the extract twice with aq. 5% KH_2PO_4, then with saturated brine. Dry the extract over anhydrous $MgSO_4$, filter the mixture, and concentrate the filtrate *in vacuo* to leave the crude title compound (1.15 g), which was used immediately in the next step.

Example 2

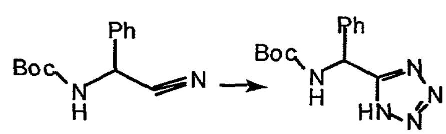
Step B.



Treat a solution of the product of the previous step (1.15 g) in pyridine (10 mL) at 5°C with $POCl_3$ (0.6 mL), then allow to warm and stir at room temperature for 3 hr. Pour the mixture onto ice (100 g), then extract with ethyl acetate (2 x 100 mL). Wash the extract with ice-cold 0.1 N H_3PO_4, then with saturated brine. Dry the extract over anhydrous $MgSO_4$, filter the mixture, and concentrate the filtrate *in vacuo* . Crystallize the residue from hexane to obtain the title compound (0.66 g, 60% yield overall).

Example 3

Step A.

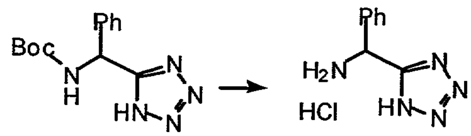


Treat a solution of the product of the previous step (0.18 g) in DMF (2 mL) with NaN_3 (0.055 g) and NH_4Cl (0.045 g), then stir at 90°C for 6 hr. Cool the reaction mixture, quench it with 10% aqueous KH_2PO_4, then extract with ethyl acetate (2 x 35 mL). Wash the extract with 10% aqueous KH_2PO_4, then with saturated brine. Dry the extract over

anhydrous $MgSO_4$, filter the mixture, and concentrate the filtrate *in vacuo* to leave the crude title compound, which was used in the next step without further purification; $C_{13}H_{17}N_5O_2$ (275.31); LRMS (FAB) M+1 = 276.2.

Example 3

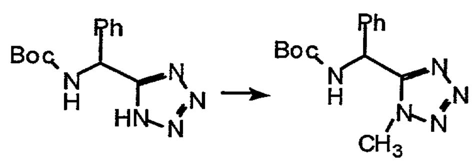
Step B



Use the procedure of Example 1 Step C. above to treat the product of the previous step to obtain the product as a white powder, which is used subsequently without further purification.

Example 4

Step A.



4A

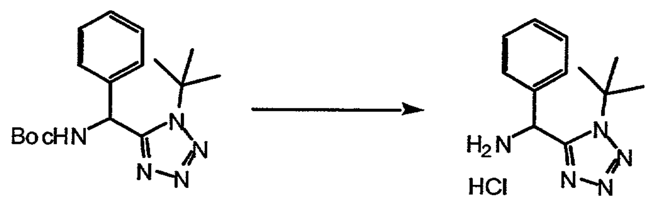
Treat a solution of the product of Example 2a. (0.055 g) and THF (1.5 mL) at 5°C with excess of a solution of diazomethane in Et_2O. Allow the solution to warm to room temperature over 2 hr., quench with hexane, and concentrate the filtrate *in vacuo* to leave the crude title compound (0.056 g), which was used without further purification; $C_{14}H_{19}N_5O_2$ (Mol. Wt.: 289.33), LRMS (FAB) M+1 = 290.0.

Example 4

Step B.

Use the procedure if Example 1 Step C. above to treat the product of the preceding step (0.055 g) to obtain the product as a white powder (0.027 g), as a 3:1 mixture of regioisomers, $C_9H_{11}N_5 \cdot HCl$ (225.68) H1NMR (DMSO-d6) d 9.3 (br s, 3 H), 7.45 (m, 5 H), 6.22 (s, 0.3 H) and 6.03 (s, 0.7 H), 4.39 (s, 2.1 H) and 3.94 (s, 0.9 H).

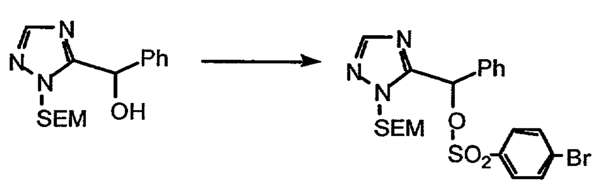
Example 5



Following the procedure of Example 1, Step C above, the product of the previous Step was converted to the corresponding product, which is used subsequently without further purification.

Example 6

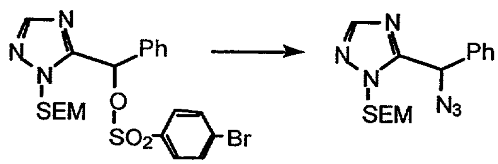
Step A



4-bromobenzenesulfonyl chloride (7.1 g) was added to a solution of the ethyl alcohol (N. Fugina, et al., Heterocycles,. ***1992***, 34(2), 303-314) at 0°C, followed by Et_3N (3.9 mL) and DMAP (3.4 g), and stir the mixture for 18 hr. at ambient temperature. Wash the reaction mixture with 10%

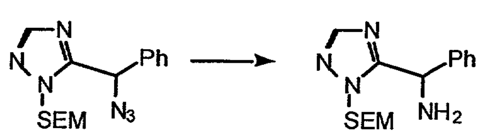
aqueous KH_2PO_4, then brine, and dry the solution over anhydrous $MgSO_4$. Filter the mixture, evaporate solvent *in vacuo*, and chromatograph the residue on silica gel (15:85 EtOAc-CH_2Cl_2) to obtain the product (3.6 g) $C_{21}H_{26}BrN_3O_4SSi$ (524.51) LRMS (FAB) M+H= 524.2.

Example 6
Step B



Stir a mixture of the product from the step above (3.6 g), sodium azide (0.56 g), and DMF (50 mL) at 100°C for 4 hr. Pour the cooled reaction mixture into cold water, extract with EtOAc, wash the extract with brine, and dry it over anhydrous $MgSO_4$. Filter the mixture, evaporate solvent *in vacuo*, and chromatograph the residue on silica gel (3:97 EtOAc-CH_2Cl_2) to obtain the product (2.8 g) $C_{15}H_{22}N_6OSi$ (330.47) LRMS (FAB) M+H= 331.2.

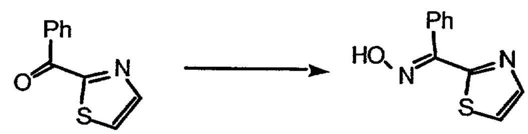
Example 6
Step C



Treat a solution of the product from the step above (1.3 g) in EtOH (50 mL) with 10% Pd-C (0.15 g) and hydrogen at 1 atm. for 18 hr. Filter

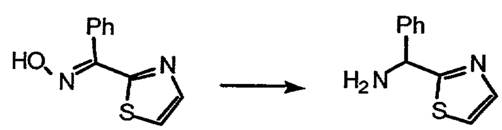
the mixture and evaporate solvent *in vacuo* to leave the product (1.2 g) $C_{15}H_{24}N_4OSi$ (304.47) LRMS (FAB) M+H= 305.3.

Example 7
Step A



A stirred solution of 2-benzoylthiazole (1.9 g, G. Jones, et al., *Tetrahedron*, **1991**, *47* (16), 2851-2860.) in EtOH:H_2O (50:5 mL) was treated with hydroxylamine hydrochloride (1.4 g), and heated at reflux for 24 hr. The cooled mixture was poured into EtOAc and washed successively with 10% aqueous KH_2PO_4, then brine. The extract was dried over anhydrous $MgSO_4$, the mixture was filtered, and the solvent was evaporated *in vacuo* to leave the product as a mixture of geometric isomers, $C_{10}H_8N_2OS$ (204.25) LRMS (FAB) M+1=205.2.

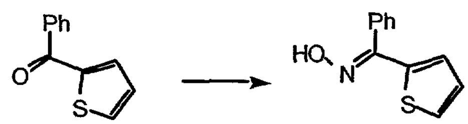
Example 7
Step B



The product of the preceding step was mixed with MeOH (30 mL), formic acid (15 mL), and water (15 mL), and cooled to 0°C. Zinc dust was added in small portions to the stirred mixture over 0.5 hr., and the mixture was stirred an additional 18 hr. at 0°C. The mixture was then suction-filtered through a celite pad, and the filtrate was evaporated *in vacuo*. The gum residue was taken up with EtOAc (0.5 L) and 1 N NaOH (0.1 L), the mixture again suction-filtered, and the aqueous layer of the filtrate

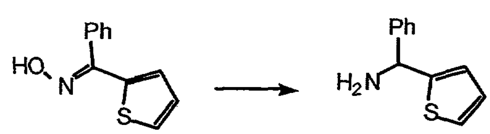
discarded. The organic extract was washed with brine and dried over anhydrous $MgSO_4$. The mixture was filtered, the solvent was evaporated *in vacuo*, and the residue was chromatographed (silica gel, 1:1 EtOAc:CH_2Cl_2) to give the product, $C_{10}H_{10}N_2S$ (190.27) LRMS (FAB) M+1=191.1.

Example 8
Step A



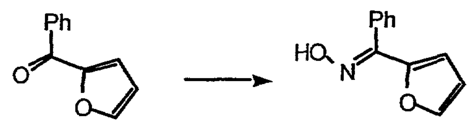
Following the procedure of Example 6 step A. above, 2-benzoylthiophene (C. Malanga, et al., *Tetrahedron Lett.*, **1995**, *36* (50), 9185-9188) was converted to the corresponding product, $C_{11}H_9NOS$ (203.26), LRMS (FAB) M+1=204.2.

Example 8
Step B



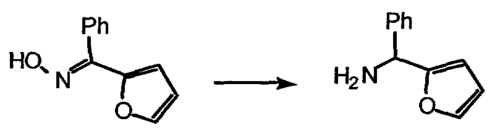
Following the procedure of Example 6 step B. above, the product of the preceding step was converted to the corresponding product, $C_{11}H_{11}NS$ (189.28), LRMS (FAB) M+1=190.2.

Example 9
Step A



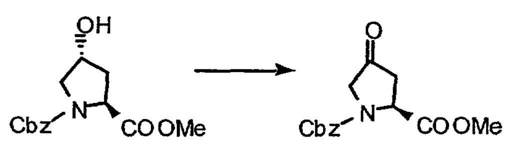
Following the procedure of Example 6 step A. above, 2-benzoylfuran (M. J. Aurell, et al., *J.Org.Chem.*, **1995**, *60* (1), 8-9) was converted to the corresponding product, $C_{11}H_9NO_2$ (187.19), 188.1.

Example 9
Step B



Following the procedure of Example 6 step B. above, the product of the preceding step was converted to the corresponding product, $C_{11}H_{11}NO$ (173.21), LRMS (FAB) M+1=174.2.

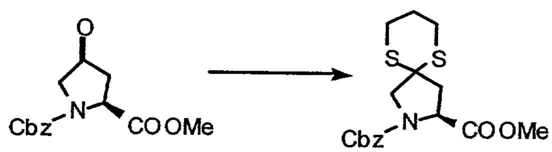
Example 10
Step A



Combine N-Cbz-hydroxyproline methyl ester (available from Bachem Biosciences, Incorporated, King of Prussia, Pennsylvania), compound (2.1) (3.0 g), toluene (30 mL), and ethyl acetate (30 mL). The mixture was stirred vigorously, and then a solution of NaBr/water (1.28 g /5 mL) was added. To this was added 2,2,6,6-tetramethyl-1-piperidinyloxy

free radical (TEMPO, 17 mg, from Aldrich Chemicals, Milwaukee, Wisconsin). The stirred mixture was cooled to 5°C and then was added a prepared solution of oxidant [commercially available bleach, Clorox® (18 mL), $NaHCO_3$ (2.75 g) and water to make up 40 mL] dropwise over 0.5 hr. To this was added 2-propanol (0.2 mL). The organic layer was separated, and the aqueous layer extracted with ethyl acetate. The organic extracts were combined, washed with 2% sodium thiosulfate, then saturated brine. The organic solution was dried over anhydrous $MgSO_4$, filtered, and evaporated the filtrate under vacuum to leave a pale yellow gum suitable for subsequent reactions (2.9 g, 97% yield), $C_{14}H_{15}NO_5$ (277.28), mass spec. (FAB) M+1 = 278.1.

Example 10
Step B

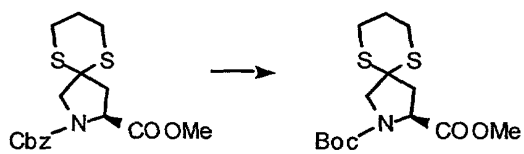


Compound (2.2) from Step A above (7.8 g) was dissolved in dichloromethane (100 mL), and cooled to 15°C. To this mixture was first added 1,3-propanedithiol (3.1 mL), followed by freshly distilled boron trifluoride etherate (3.7 mL). The mixture was stirred at room temperature for 18 h. While stirring vigorously, a solution of K_2CO_3/water (2 g / 30 mL)was carefully added, followed by saturated $NaHCO_3$ (10 mL). The organic layer was separated from the aqueous layer (pH ~7.4), washed with water (10 mL), then brine. The organic solution was dried over anhydrous $MgSO_4$, filtered, and evaporated under vacuum. The residue was chromatographed on silica gel, eluting with toluene, then a with a

gradient of hexane-Et_2O (2:3 to 0:1) to afford a brown oil (7.0 g, 68% yield), $C_{17}H_{21}NO_4S_2$ (367.48), mass spec. (FAB) M+1 =368.1.

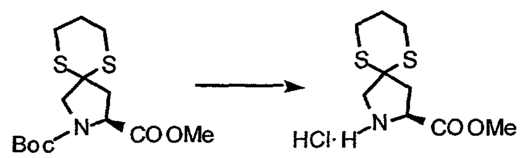
Example 10

Step C

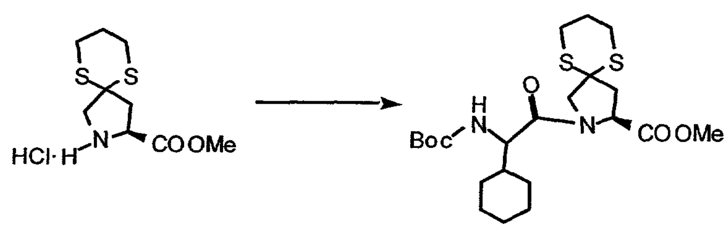


A solution of compound (2.3) from Step B above (45 g) in acetonitrile (800 mL) at 20°C was treated with freshly distilled iodotrimethylsilane (53 mL) at once. The reaction was stirred for 30 min., then poured into a freshly prepared solution of di-t-butyldicarbonate (107 g), ethyl ether (150 mL), and diisopropylethylamine (66.5 mL). The mixture stirred for 30 min. more then was washed with hexane (2 x 500 mL). Ethyl acetate (1000 mL) was added to the lower acetonitrile layer, and then the layer was washed with 10% aqueous KH_2PO_4 (2 x 700 mL), and brine. The filtrate was evaporated under vacuum in a 25°C water bath, taken up in fresh ethyl acetate (1000 mL), and washed successively with 0.1 N HCl, 0.1 N NaOH, 10% aqueous KH_2PO_4, and brine. The organic solution was dried over anhydrous $MgSO_4$, filtered, and evaporated under vacuum. The residue (66 g) was chromatographed on silica gel (2 kg), eluting with hexane (2 L), then Et_2O/hexane (55:45, 2 L), then Et_2O (2 L) to afford an orange gum which slowly crystallized on standing (28 g, 69% yield), $C_{14}H_{23}NO_4S_2$ (333.46), mass spec. (FAB) M+1 = 334.1.

Example 10
Step D



To a solution of compound (2.4) from Step C above (1 g) in dioxane (5 mL), was added 4 N HCl-dioxane solution (50 mL). The mixture was stirred vigorously for 1 hr. The mixture was evaporated under vacuum in a 25°C water bath. The residue was triturated with Et_2O, and filtered to leave the title compound (0.76 g, 93% yield), $C_9H_{15}NO_2S_2 \cdot HCl$ (269.81), mass spec. (FAB) M+1 = 234.0.



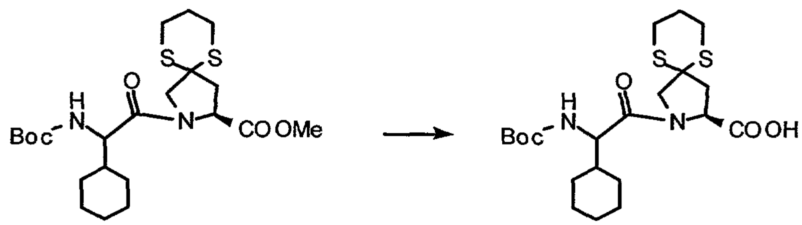
Example 10
Step E

10E

A mixture of compound (2.6) from Step E above (1.12 g), N-Boc-cyclohexylglycine (Boc-Chg-OH, 1.0 g, from Sigma Chemicals, St. Louis, Missouri), dimethylformamide (20 mL), and PyBrOP coupling reagent (2.1 g) was cooled to 5°C. To this was added diisopropylethylamine (DIEA or DIPEA, 2.8 mL). The mixture was stirred cold for 1 min., then stirred at room temperature for 6 hr. The reaction mixture was poured into cold 5% aqueous H_3PO_4 (150 mL) and extracted with ethyl acetate (2 x 150 mL). The combined organic layer was washed with cold 5% aqueous K_2CO_3,

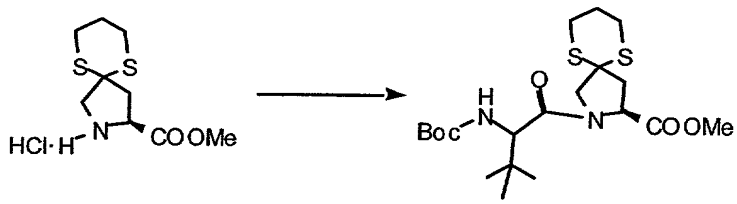
then 5% aqueous KH_2PO_4, then brine. The organic solution was dried over anhydrous $MgSO_4$, filtered, and evaporated under vacuum. The residue was chromatographed on silica gel, eluting with EtOAc-CH_2Cl_2 to afford a white solid (0.8 g, 50% yield), $C_{22}H_{36}N_2O_5S_2$ (472.66), mass spec. (FAB) M+1 =473.2.

<u>Example 10</u>
<u>Step F</u>



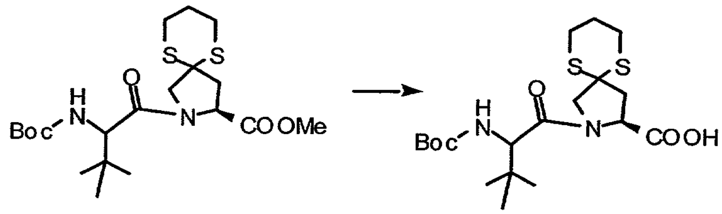
A solution of compound (?) from Step ? above (0.8 g) in dioxane (10 mL) at 20°C was treated with 1N aqueous LiOH (3.4 mL) and stirred for 4 h. The mixture was concentrated under vacuum in a 30°C water bath to half volume. The remainder was diluted with water (25 mL), extracted with Et_2O (2 x 20 mL). The aqueous layer was acidified to pH ~4 with 6 N HCl, extracted with ethyl acetate, and washed with brine. The organic solution was dried over anhydrous $MgSO_4$, filtered, and evaporated under vacuum to leave the title compound (2.8) (0.7 g), $C_{21}H_{34}N_2O_5S_2$ (458.64), mass spec. (FAB) M+1 =459.2.

Example 12
Step A



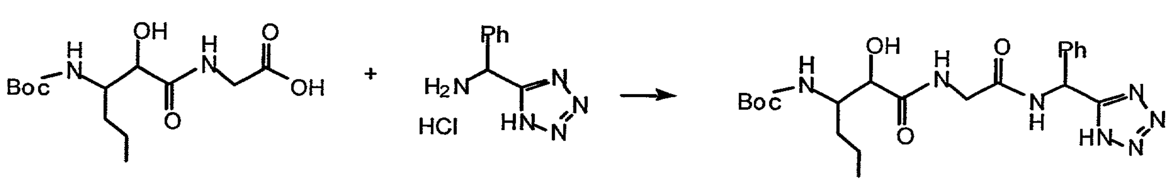
Following the procedure of Example 10 step E. above, N-Boc-Tle-OH (Bachem Biosciences, Inc.) and the product of Example 9 step D. were reacted to give the corresponding product, $C_{20}H_{34}N_2O_5S_2$ (446.63), LRMS (FAB) M+1 =447.3.

Example 12
Step B



Following the procedure of Example 10 step E. above, the product of the preceding step was converted to the corresponding product, $C_{19}H_{32}N_2O_5S_2$ (432.60), LRMS (FAB) M+1 =433.3.

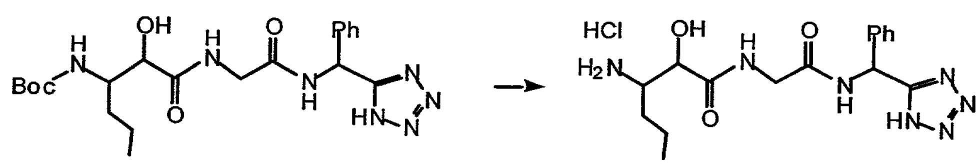
Example 12
Step A



Cool a stirred mixture of the product of the previous step (0.11 g), the product of Example-1 Step E. above [Boc-Nva(OH)-Gly-OH] (0.205 g), dimethylformamide (7 mL), and PyBrOP coupling reagent (0.385 g) to 5°C, then add diisopropylethylamine (DIPEA, 0.252 mL). Stir the mixture cold for 1 min., then stir at room temperature for 6 hr. Pour the reaction mixture into cold 1% aqueous H_3PO_4 (150 mL) and extract with ethyl acetate. Wash the combined organics with cold 5% aqueous K_2CO_3, then 5% aqueous KH_2PO_4, then brine. Dry the organic solution over anhydrous $MgSO_4$, filter, and evaporate the filtrate *in vacuo* to leave the crude title compound (0.15 g), which was used in the next step without further purification.

Example 12

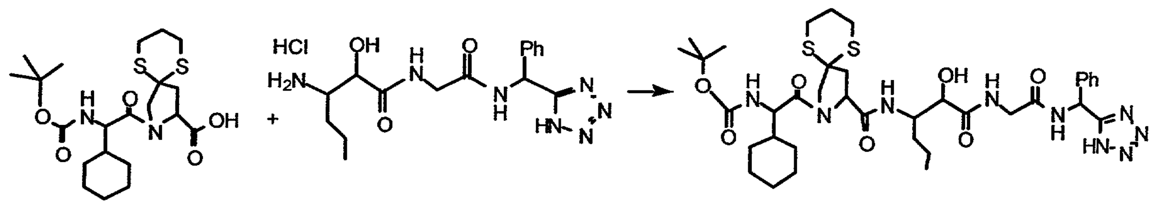
Step B



Treat the product of the previous step with a 4N solution of HCl in dioxane for 0.5 hr. concentrate the filtrate *in vacuo* in a 30°C water bath, and tritrate the residue with Et_2O. Filter the mixture to leave the title compound as a white powder 90.13 g0, which was used in the next step without further purification; $C_{16}H_{23}N_7O_3$ (361.40), LRMS (FAB) M+1 = 362.4.

Example 12

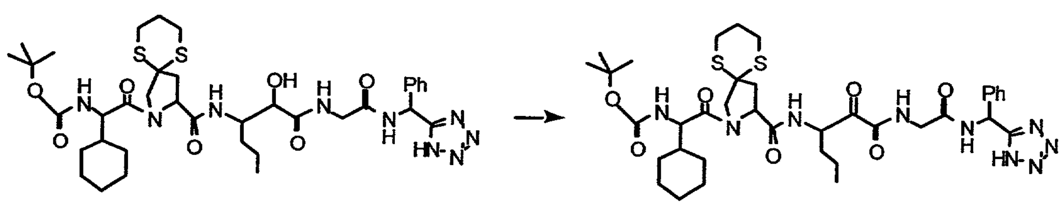
Step C



Cool a stirred mixture of the product of Example 5 step B. (0.06 g), the product of Example-8 Step G. (0.85 g), dimethylformamide (8 mL), and PyBrOP coupling reagent (0.088 g) to 5°C, then add diisopropylethylamine (DIPEA, 0.89 mL). Stir the mixture cold for 1 min., then stir at room temperature for 48 hr. Pour the reaction mixture into cold 1% aqueous H_3PO_4 and extract with ethyl acetate. Wash the combined organics with 5% aqueous KH_2PO_4, then brine. Dry the organic solution over anhydrous $MgSO_4$, filter, and evaporate the filtrate *in vacuo* to leave the crude title compound (0.13 g). Chromatograph the residue on silica gel with MeOH-CH_2Cl_2 (1:99 to 10:90 gradient) to obtain the title compound (0.092 g); $C_{37}H_{55}N_9O_7S_2$ (802.02), LRMS (FAB) M+1 = 802.6.

Example 12

Step D

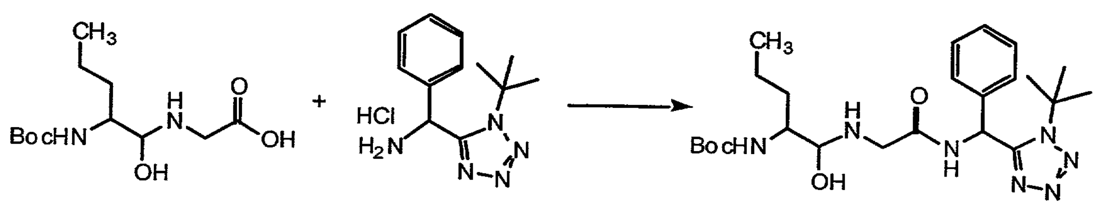


Cool a solution of oxalyl chloride (25 μL) and CH_2Cl_2 to -70°C. Add slowly a solution of methyl sulfoxide (DMSO, 50 μL) and CH_2Cl_2 (1 mL) below -60°C. Cool to -70°C, and add dropwise a solution of the product of

the previous step (0.0.09 g) and CH_2Cl_2 (1 mL) below -60°C. Stir an additional 0.5 hr., add slowly triethylamine (Et_3N, 0.13 mL) below -50°C, then warm to 10°C. Dilute the reaction with excess ethyl acetate, and wash the solution with cold 0.1 N HCl, then brine. Dry the organic solution aver anhydrous $MgSO_4$, filter, and evaporate the filtrate under vacuum. Chromatograph the residue on silica gel, eluting with MeOH-CH_2Cl_2 (1:99 to 25:75 gradient) to obtain the title compound (0.011 g, 12% yield), $C_{37}H_{53}N_9O_7S_2$ (800.01), LRMS (FAB) M+1 = 800.3.

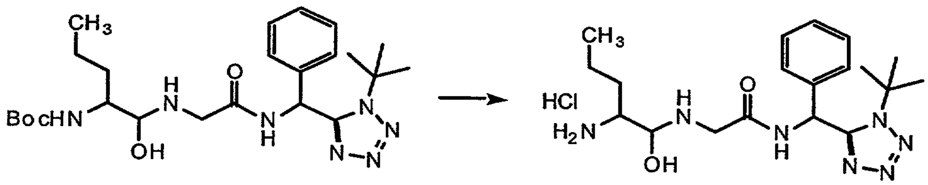
Example 13

Step A



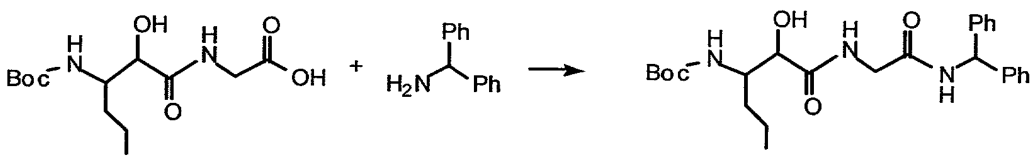
A solution of the product of example 1 step E. (100 mg, 0.22 mmols) in dry DMF (2.5 mL) was treated with HOOBt (45 mg, 0.33 mmols) and Hünigs base (141 mg, 1.1 mmols, 5.0 equiv.). The reaction mixture was cooled to -20° C and treated with EDCI (63 mg, 0.33 mmols, 1.5 equiv) and stirred for 20 min. The reaction mixture was treated with amine hydrochloride (118 mg, 0.27 mmols, 1.22 equiv.) and stirred at rt for 12 h. The reaction mixture was concentrated in vacuo and diluted with H_2O (30 mL). The aqueous layer was extracted with CH_2Cl_2 (3x50 mL) and EtOAc (3x50 mL). The combined organic layers were extracted with aq. HCl (2M), aq. $NaHCO_3$ (satd), dried ($MgSO_4$) filtered concentrated in vacuo to obtain a colorless solid **1k** (79 mg) which was used for oxidation; LRMS [electron spray, *m/z (rel int)*]: M+1 = 826 (100).

Example 13
Step B



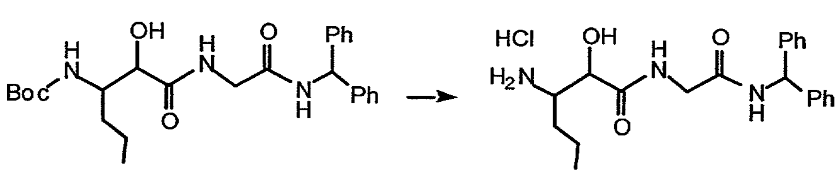
Following the procedure of Example 12 Step B., the product of the preceding Step was converted to the corresponding product, which was used as it was for subsequent reactions. MS (Electron spray): [835 $(2M+1)^+$, 40], 418 $[M+1)^+$, 100)].

Example 14
Step A



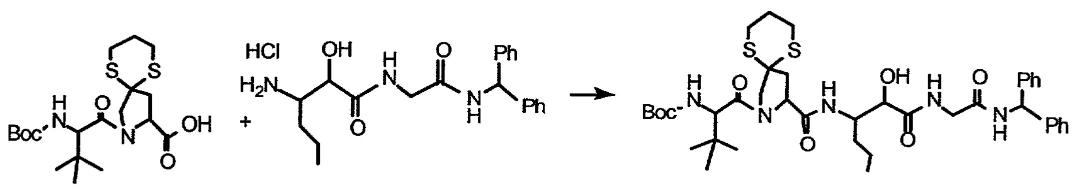
Following the procedure of Example 12 Step A. above, the product of Example 1 step E. is reacted with benzhydrylamine to give the corresponding product, $C_{26}H_{35}N_3O_5$ (469.57), LRMS (FAB) M+1=470.4.

Example 14
Step B



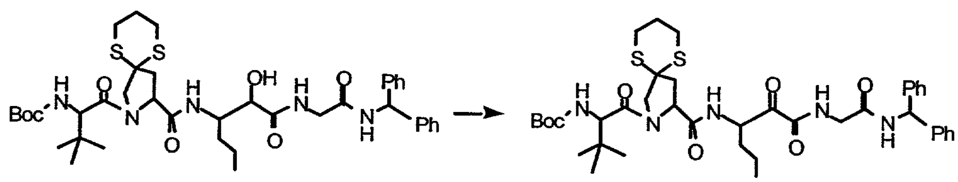
Following the procedure of Example 12 step B., the product of the preceding step was converted to the corresponding product, $C_{21}H_{27}N_3O_3 \cdot HCl$ (405.92), LRMS (FAB) M+1= 370.4.

Example 14
Step C



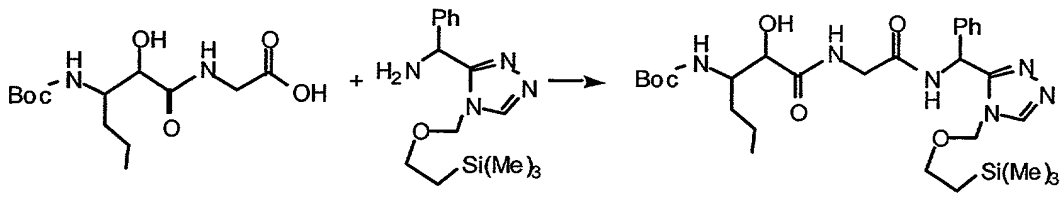
Following the procedure of Example 12 step C., the product of the preceding step was reacted with the product of Example 10 step B. to give the corresponding product, $C_{40}H_{57}N_5O_7S_2$ (784.04), LRMS (FAB) M+1= 784.5.

Example 14
Step D



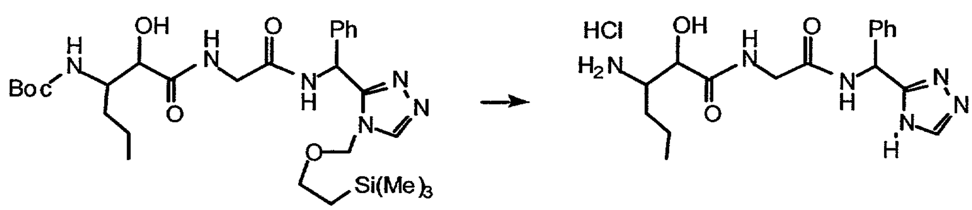
Following the procedure of Example 12 step D., the product of the preceding step was converted to the corresponding product, $C_{40}H_{55}N_5O_7S_2$ (782.03), LRMS (FAB) M+1= 782.4.

Example 15
Step A



Following the procedure of Example 12 Step A. above, the product of Example 1 step E. is reacted with the product of Example 5 step C. to give the corresponding product, $C_{28}H_{46}N_6O_6Si$ (590.79), LRMS (FAB) M+1= 591.4.

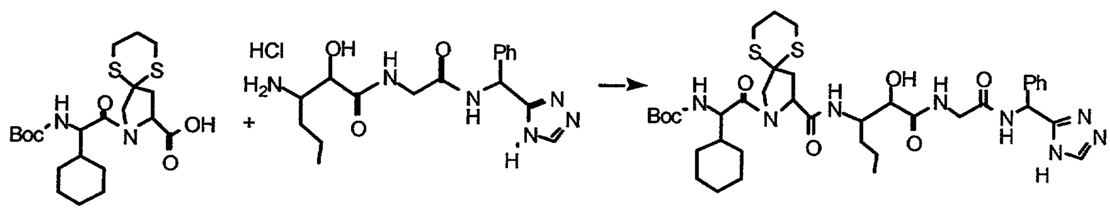
Example 15
Step B



Following the procedure of Example 12 Step B., the product of the preceding step was converted to the corresponding product, $C_{17}H_{24}N_6O_3 \cdot HCl$ (396.87), LRMS (FAB) M+1= 361.3.

Example 15

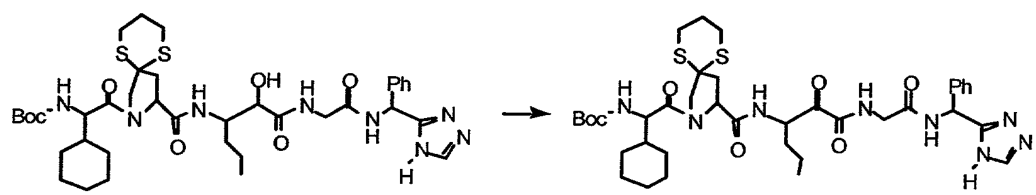
Step C



Following the procedure of Example 12 Step C., the product of the preceding step was converted to the corresponding product, $C_{38}H_{56}N_8O_7S_2$ (801.03), LRMS (FAB) M+1= 801.5.

Example 15

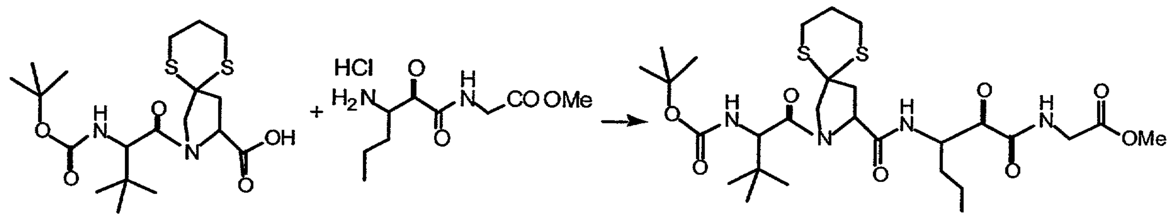
Step D



Following the procedure of Example 12 Step D., the product of the preceding step was converted to the corresponding product, $C_{38}H_{54}N_8O_7S_2$ (809.02), LRMS (FAB) M+1= 799.4.

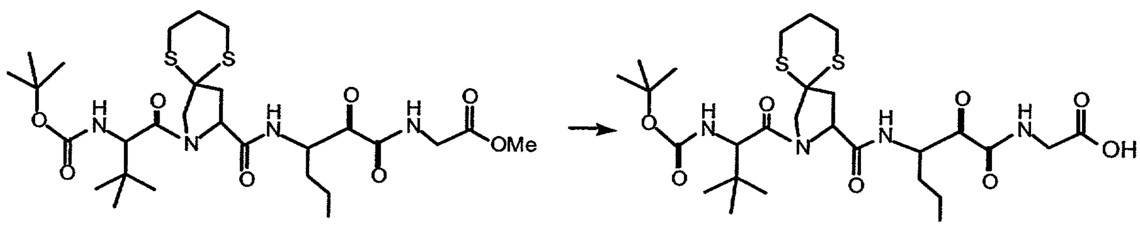
Example 16

Step A



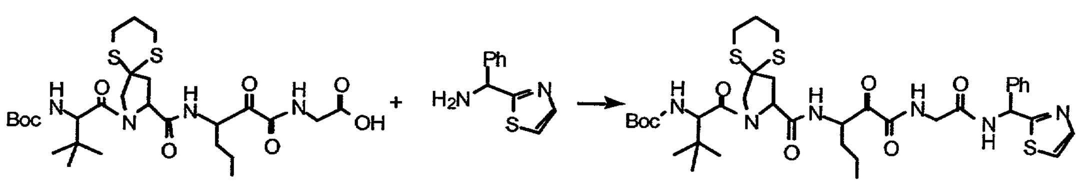
Follow the procedure of Example 1A but use the acid of Example Step 10B above and the amine of Example Step 1C to obtain the compound, $C_{28}H_{46}N_4O_8S_2$ (630.82) LRMS (FAB) M+H = 631.4.

Example 16
Step B



Follow the procedure of Example 1 Step E but use the ester of the preceding Step to obtain the compound, $C_{27}H_{44}N_4O_8S_2$ (616.79) LRMS (FAB) M+H= 617.4.

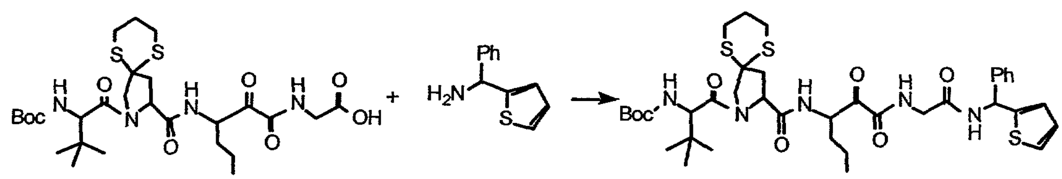
Example 16
Step C



A stirred mixture of the product of the preceding Step (62 mg), the product of Example 7 Step D. (29 mg), HATU (57 mg, *O*-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate, Aldrich Chemical Co.) and CH_2Cl_2 (5 mL) at 0°C was treated with diisopropylethylamine (.023 mL), and the mixture was stirred an additional 3 hr. at room temperature. The mixture was poured into ice-cold EtOAc

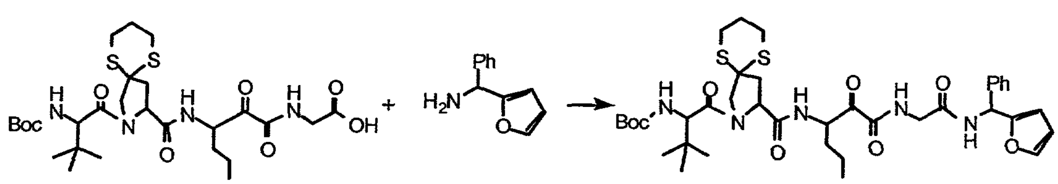
(50 mL) and washed successively with cold 5% aqueous K_2CO_3, cold 0.1 N HCl, and brine. The extract was dried over anhydrous $MgSO_4$, the mixture was filtered, the filtrate was evaporated *in vacuo*, and the residue was chromatographed (silica gel, 1:1 EtOAc:CH_2Cl_2). The crude product was triturated under (*i*-Pr)20 and filtered to leave the product as a white powder (81 mg), $C_{37}H_{52}N_6O_7S_3$ (789.04), LRMS (FAB) M+1 = 789.4.

Example 17



Following the procedure of Example of Example 16 Step C., the product of Example 16 Step B. was reacted with the product of Example 9 Step B. to obtain the corresponding product, $C_{38}H_{53}N_5O_7S_3$ (788.05), LRMS (FAB) M+1 = 788.4.

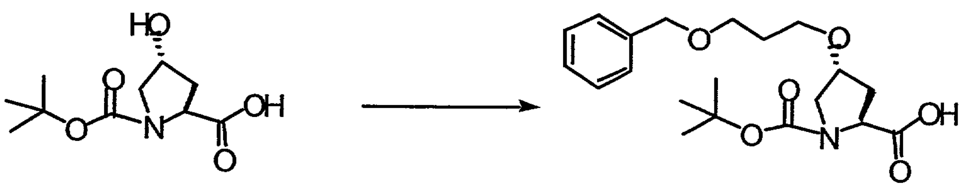
Example 18



Following the procedure of Example of Example 16 Step C., the product of Example 16 Step B was reacted with the product of Example 9 Step B to obtain the corresponding product, $C_{38}H_{53}N_5O_8S_2$ (771.99), LRMS (FAB) M+1 = 772.4.

Example 19

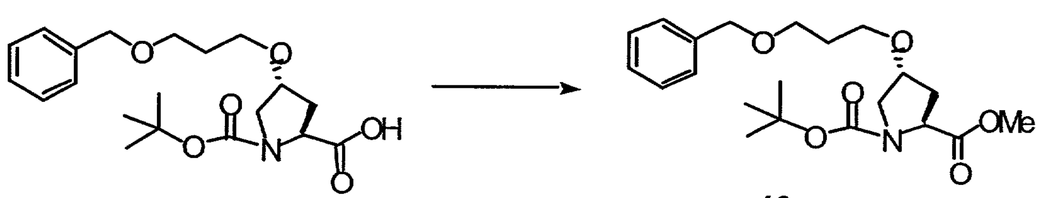
Step A:



19a 19b

To a solution of Boc-Hyp-OH (7.0 g, 30.3 mmol) and benzyl 3-bromopropyl ether (7.8 g, 34.0 mmol) in anhydrous DMF (400 mL) at room temperature was added sodium hydride (3.5 g, 60% dispersion in mineral oil, 87.5 mmol) and sodium iodide (0.5 g, 3.33 mmols) with stirring. The resulting suspension was vigorously stirred at room temperature overnight (18 h). The reaction was quenched carefully with a slow addition of water (50 mL) and acidified with 6 N HCl solution (20 mL). After addition of ethyl acetate (800 mL), brine (150 mL) and more water (150 mL), the formed two layers were separated and the organic layer was washed with 5% H_3PO_4 (3X200 mL). It was then dried with $MgSO_4$, filtered and concentrated in vacuo to afford **19b** as an oil which was used in Step B without further purification.

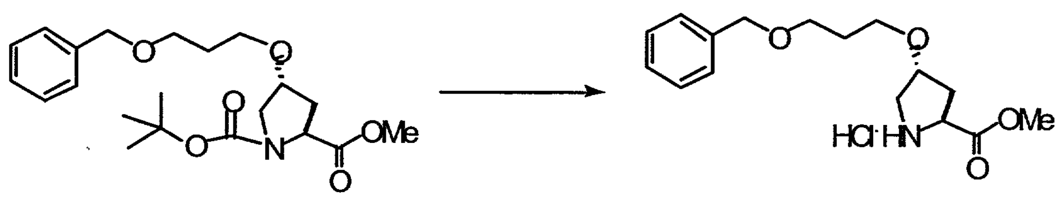
Step B:



19b 19c

The acid **19b** from Step A was dissolved in benzene (25 mL) and methanol (28 mL). To this solution at room temperature was added a solution of trimethylsilyl diazomethane (27 mL, 2.0 M in cyclohexane) with caution. After being stirred at room temperature for 1 h, it was concentrated in vacuo to yield the methyl ester. Flash chromatography (8 to 20 % EtOAc-CH_2Cl_2) afforded **1c** (5.15 g; 13.1 mmol, 43%, 2 steps) as an oil.

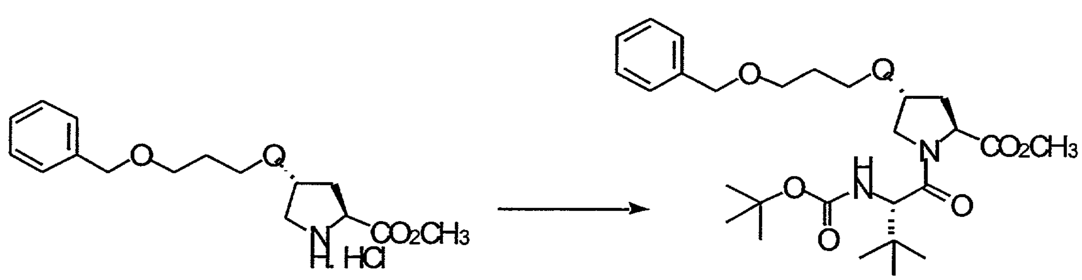
Step C:



19c **19d**

The Boc-amino methyl ester **19c** (5.83 g, 14.8 mmol) was dissolved in 4 N HCl in dioxane (80 mL, 320 mmol) and the resulting solution was stirred at room temperature. The progress of the reaction was monitored by TLC. After 5 h, the solution was concentrated in vacuo and the residue was kept under vacuum overnight to yield a white solid which was used in the next coupling reaction without further purification.

Step D

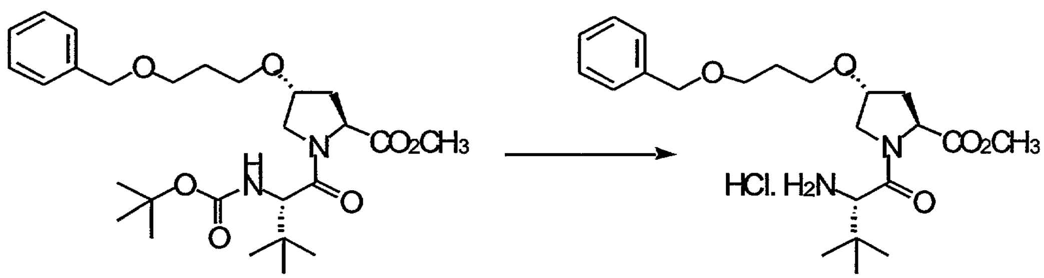


19d **19e**

To a solution of the amine ester **19d** (from Step 19B), *N*-Boc-tertbutylglycines as coupling partners 14.9 mmol), HOOBt (2.60 g, 15.9 mmol) and EDCI (3.41 g, 17.8 mmol) in anhydrous DMF (150 mL) and CH_2Cl_2 at -20^{0}C, was added NMM (6.50 mL, 59.1 mmol). After being stirred at this temperature for 30 min, the reaction mixture was kept in a freezer overnight (18 h). It was then stirred in air and allowed to warm to room temperature in 1h. EtOAc (450 mL), brine (100 mL) and 5% H_3PO_4 (100 mL) were added. The separated organic solution was washed with 5% H_3PO_4 (100 mL), saturated aqueous sodium bicarbonate solution (2 X 150 mL), water (150 mL), and brine (150 mL), dried with magnesium sulfate, filtered and concentrated in vacuo.

The material of **19e** was purified by flash column chromatography using 90/10 dichloromethane/ethyl acetate to provide **12a** in 73% yield. ^{13}C NMR (mixture of rotamers, $CDCl_3$) 26.20, 28.31, 29.07, 30.06, 34.94, 35.86, 37.06, 51.21, 52.16, 52.84, 57.78, 58.33, 65.95, 66.92, 72.97, 75.48, 79.45, 127.55, 127.66, 128.35, 138.45, 155.62, 165.06, 171.13, 172.54; HRMS (FAB) Calcd for $C_{27}H_{43}N_2O_7$: 507.3070 $(M+H)^+$. Found: 507.3077.

Step E:

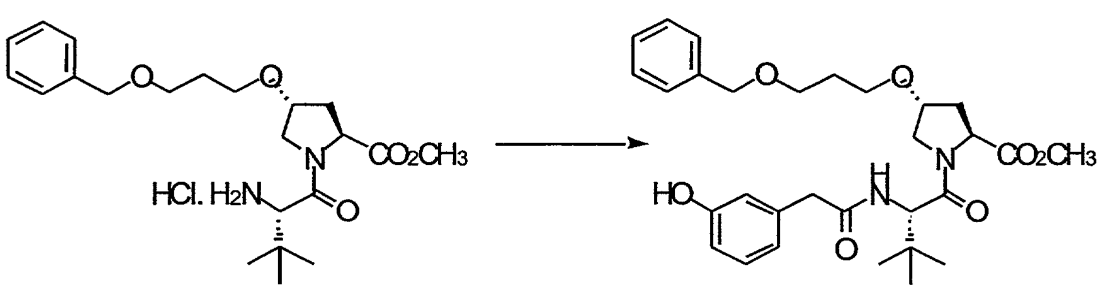


19e 19f

The desired compound **19f** was prepared as follows

The Boc-amino methyl ester **19e** (6.53 g, 12.3 mmol) was dissolved in 4 N HCl (60 mL, 240 mmol) and the resulting solution was stirred at room temperature. The progress of the reaction was monitored by TLC. After 4 h, the solution was concentrated in vacuo and the residue was kept under vacuum overnight to give a white solid which was used in the next coupling reaction without further purification. The material was carried forward to the next step.

Step F:



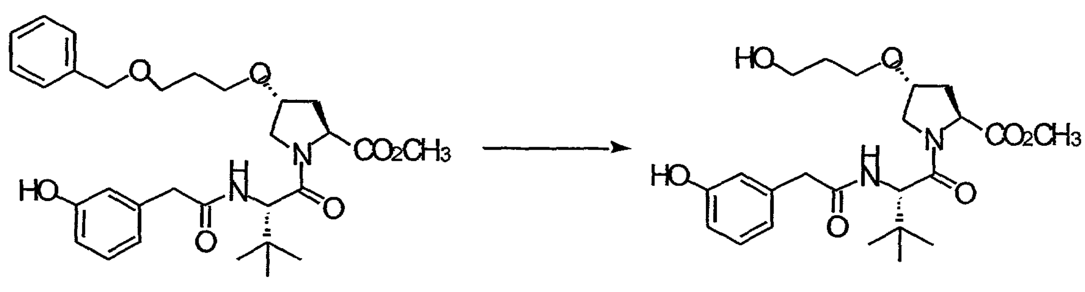
19f 19g

The desired product **19g** was prepared as follows:

To a solution of the amine **19f** (from Step 1D), 3-hydroxy phenylacetic acid (1.90 g, 12.5 mmol), HOOBt (2.10 g, 12.9 mmol) and EDCI (2.85 g, 14.9 mmol) in anhydrous DMF (250 mL) and CH_2Cl_2 (100 mL) at -20^{0}C, was added NMM (4.20 mL, 38.2 mmol). After being stirred at this temperature for 30 min, the reaction mixture was kept in a freezer overnight (18 h). It was then stirred in air and allowed to warm to room temperature in 1h. EtOAc (500 mL), brine (100 mL) and 5% H_3PO_4 (100 mL) were added. The separated organic solution was washed with 5% H_3PO_4 (100 mL), saturated aqueous sodium bicarbonate solution (2 X 150 mL), water (150 mL), and brine (150 mL), dried with magnesium sulfate, filtered and concentrated in vacuo.

The material was purified by flash column chromatography using 99/1 dichloromethane/methanol to yield **19g** in 91%. ^{13}C NMR ($CDCl_3$) δ 26.24, 29.93, 34.95, 35.96, 43.48, 52.18, 53.09, 57.06, 58.06, 66.10, 66.92, 72.93, 77.43, 114.59, 116.14, 120.87, 127.58, 127.64, 127.74, 128.37, 130.02, 135.95, 138.39, 156.90, 170.65, 171.06, 172.38; HRMS (FAB) Calcd for $C_{30}H_{41}N_2O_7$: 541.2914 $(M+H)^+$. Found: 541.2921.

Step G:



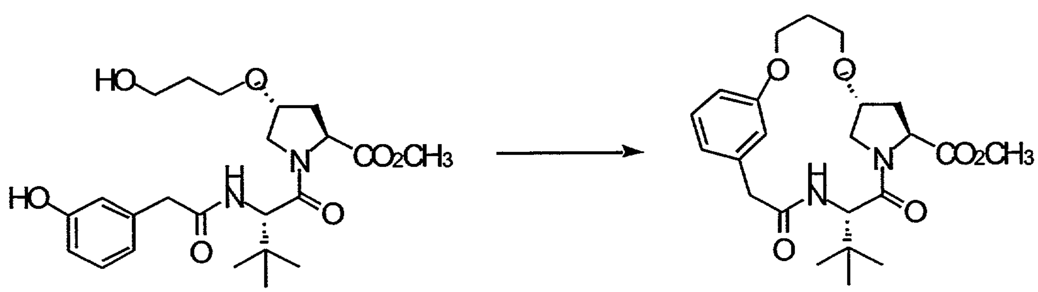
19g **19h**

The desired product **19h** was prepared as follows:

To a solution of the benzyl ether **19g** (6.23 g, 11.0 mmol) in ethanol (200 mL) under nitrogen at room temperature was added 10 % Pd-C (1.5 g) cautiously. The resulting suspension was vigorously stirred at room temperature under hydrogen for 23 h.

The product **19h** obtained after filtering off the catalyst was pure enough for subsequent manipulations. ^{13}C NMR ($CDCl_3$) δ 26.27, 32.09, 35.44, 35.67, 43.19, 52.21, 52.74, 57.60, 58.21, 58.75, 65.78, 77.74, 114.74, 116.02, 120.68, 130.07, 135.66, 157.11, 170.59, 172.05, 172.51; HRMS (FAB) Calcd for $C_{23}H_{35}N_2O_7$: 451.2444 $(M+H)^+$. Found: 451.2436.

Step H:



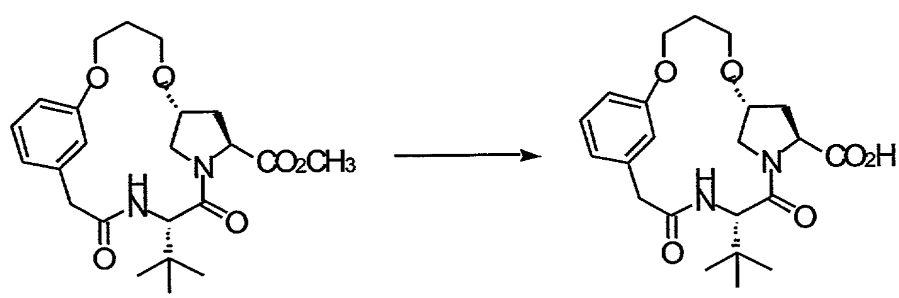
19h **19i**

The desired product **19i** was prepared as follows:

A solution of the phenol alcohol (9.43 mmol) and ADDP (6.60 g, 26.2 mmol) in anhydrous CH_2Cl_2 was bubbled with argon through a frit glass bubbler for 20 min. To this solution at 0^oC was added triphenylphosphine (4.10 g, 16.3 mmol). After stirring at 0°C for 20 min, a second portion of triphenylphosphine (3.40 g, 13.5 mmol) was added. The solution was then warmed to room temperature and stirred overnight (24 h) under nitrogen until TLC indicated the complete consumption of the starting material.

The crude material was suspended in ethyl acetate/hexane (approx. 1/1) and the undissolved solid material was filtered off. Repeated this process once again, the filtrate was concentrated and applied on the column as a dichloromethane solution. The column was eluted with 75/25 hexane/acetone to yield 29% of **19i**. HRMS (FAB) Calcd for $C_{23}H_{33}N_2O_6$: 433.2339 $(M+H)^+$. Found: 433.2339.

Step I:



19i **19j**

The desired compound **19j** was prepared as follows in quantitative yields:

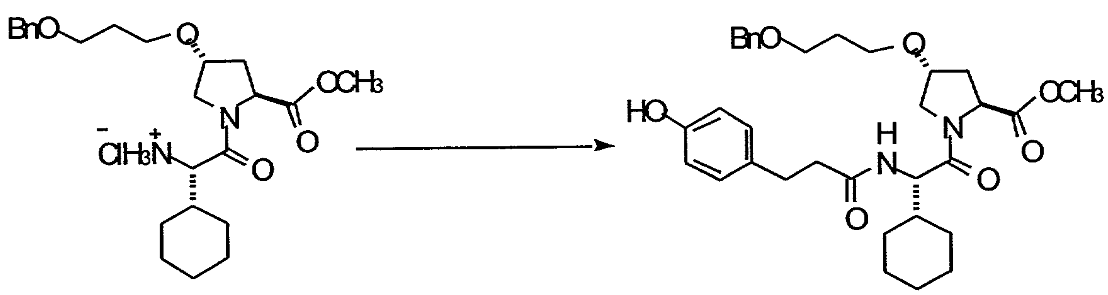
An aqueous lithium hydroxide solution (0.45 g in 30 mL H_2O) was added to a 0°C solution of the methyl ester **19j** in THF (30 mL) and methanol (30 mL). The mixture was stirred in an ice bath and warmed to room temperature along with it in 4 h. The progress of the reaction was monitored by TLC. After the volatiles were removed in vacuo, EtOAc (150 mL) and water (30 mL) were added and the two layers separated. The aqueous solution was extracted again with CH_2Cl_2 (150 mL), after which it was acidified to pH = 1. EtOAc (200 mL) was then added and the aqueous solution was saturated with solid sodium chloride. After separation of the layers, the aqueous layer was extracted with EtOAc (2 X 150 mL). Organic

solutions were combined, dried with magnesium sulfate, filtered and concentrated in vacuo to afford compound **19j**.

^1H NMR (DMSO-d6) δ 0.96 (s, 9H), 1.66-1.70 (m, 1H), 1.75-1.82 (m, 2H), 2.43 (dd, 1H), 3.32-3.36 (m, 2H), 3.48-3.52 (m, 1H), 3.55 (dd, 1H), 3.84 (app. d, 1H), 3.99 (app. d, 1H), 4.06-4.10 (m, 3H), 4.16 (dd, 1H), 4.69 (d, 1H), 6.70-6.72 (m, 3H), 7.15 (app. t, 1H), 8.42 (d, 1H), 12.43 (br. s, 1H); ^{13}C NMR (DMSO-d$_6$) δ 26.25, 28.54, 33.31, 34.97, 41.22, 53.96, 56.11, 56.97, 63.36, 64.96, 76.84, 111.94, 115.25, 121.73, 129.13, 138.36, 158.27, 169.85, 170.15, 173.04; HRMS (FAB) Calcd for $C_{22}H_{31}N_2O_6$: 419.2182 $(M+H)^+$. Found: 419.2180.

Example 20

Step A



20a **20b**

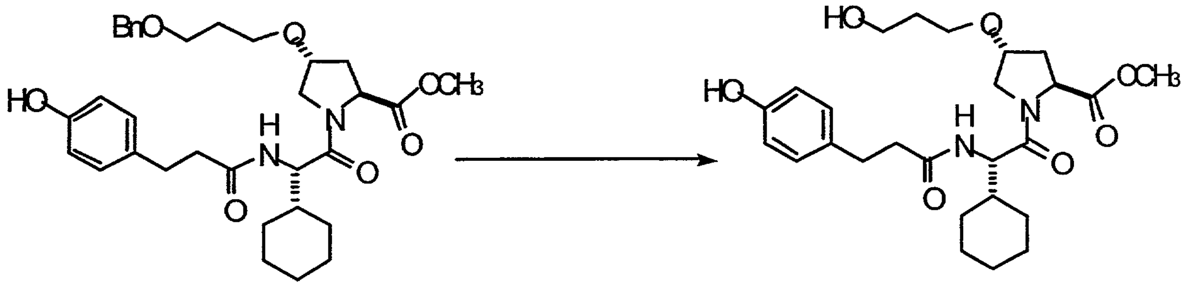
The compound **20a** was prepared as set forth in Scheme 9 referencing to Scheme 8.

The desired product **20b** was prepared as follows:

To a solution of the amine **20a**, 3-hydroxy phenylacetic acid (1.90 g, 12.5 mmol), HOOBt (2.10 g, 12.9 mmol) and EDCl (2.85 g, 14.9 mmol) in anhydrous DMF (250 mL) and CH_2Cl_2 (100 mL) at -20^{0}C, was added NMM (4.20 mL, 38.2 mmol). After being stirred at this temperature for 30 min, the reaction mixture was kept in a freezer overnight (18 h). It was then stirred in air and allowed to warm to room temperature in 1h. EtOAc (500 mL), brine (100 mL) and 5% H_3PO_4 (100 mL) were added. The separated organic solution was washed with 5% H_3PO_4 (100 mL), saturated aqueous sodium bicarbonate solution (2 X 150 mL), water (150 mL), and brine (150 mL), dried with magnesium sulfate, filtered and concentrated in vacuo.

The material was purified by flash column chromatography using EtOAc/Hex (7:3) to yield **64a** in 80%.; ^{1}H NMR ($CDCl_3$, δ) : 7.35-7.29 (m, 5 H), 7.02 (d, 2 H, *J*=8.4 Hz), 6.72 (d, 2 H, *J*= 6.9Hz) 6.01 (d, 1 H), 4.60 (t, 1 H), 4.52 (s, 1 H), 3.8-3.61(m, 2 H), 3.72 (s, 3 H), 3.54-3.51(m, 4 H), 2.83 (t, 2 H, *J*=7.5 Hz), 2.39 (t, 2 H, *J*= 8.1 Hz) 2.41-2.20 (m, 1 H), 2.05-1.83 (m, 1 H), 1.85-1.58 (m, 8 H), 1.26-1.24 (m, 5 H); ^{13}C NMR ($CDCl_3$, δ): 172.2, 171.9, 171.0, 154.4, 138.3, 132.2, 129.4, 128.4, 127.7, 127.6, 115.4, 73.0, 66.9, 66.2, 57.9, 54.9, 52.5, 52.3, 41.0, 38.5, 34.7, 30.8, 30.0, 29.4, 27.9, 26.1, 26.0, 25.9.

Step B

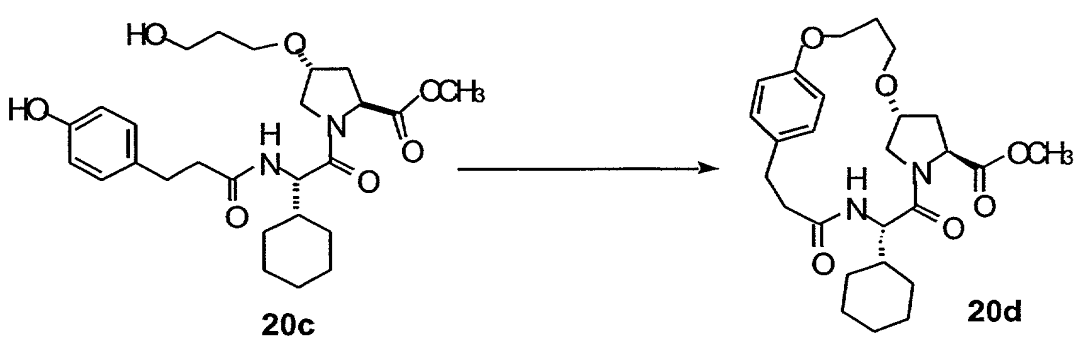


20b 20c

The desired product **20c** was obtained as follows:

To a solution of **20c** (11.0 mmol) in ethanol (200- ml) under nitrogen at room temperature was added 10% Pd/C (1.5g) cautiously. The resulting suspension was vigorously stirred at room temperature under hydrogen for 23h.

Step C



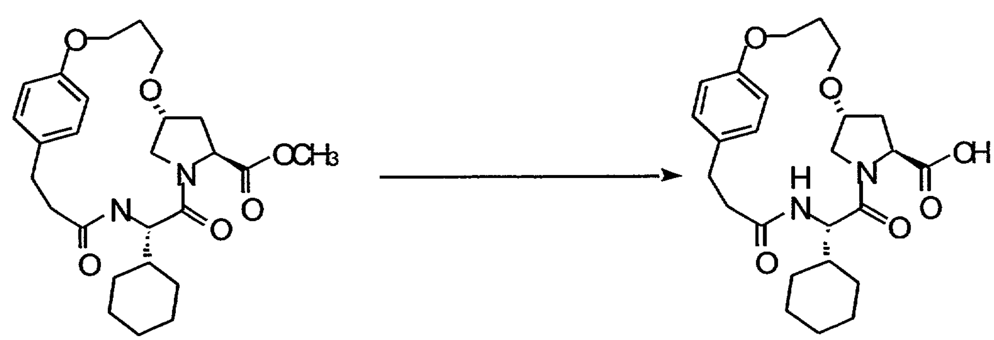
20c 20d

The desired product **20d** was obtained as follows:

A solution of **20d** (9.43 mmol) and ADDP (6.60g, 26.2 mmol) in anhydrous CH_2Cl_2 was bubbled with argon through a frit glass bubbler for 20 min. To this solution at 0°C was added triphenylphosphine (4.10g, 16.3 mmol). After stirring at 0°C for 20 min, a second portion of triphenylphosphine (3.40g, 13.5 mmol) was added. The solution was then warmed to room temperature and stirred overnight (24h) under nitrogen until TLC indicated the complete consumption of the starting material.

The crude reaction mixture was purified by SiO_2 gel chromatography (acetone/Hexanes 3:7) to yield **64c** (64 mg, 16%) as a colorless solid.; ^{13}C NMR ($CDCl_3$) δ 172.1, 171.1, 171.0, 157.7, 131.0 129.9, 114.3, 78.1, 64.7, 63.3, 58.7, 55.3, 52.2, 52.0, 42.1, 37.9, 36.1, 30.8, 30.7, 29.7, 28.7, 28.5, 26.2, 26.0; MS (FAB) 473 $(M+1)^+$, (100), 327 (20).

Step D



20d **20e**

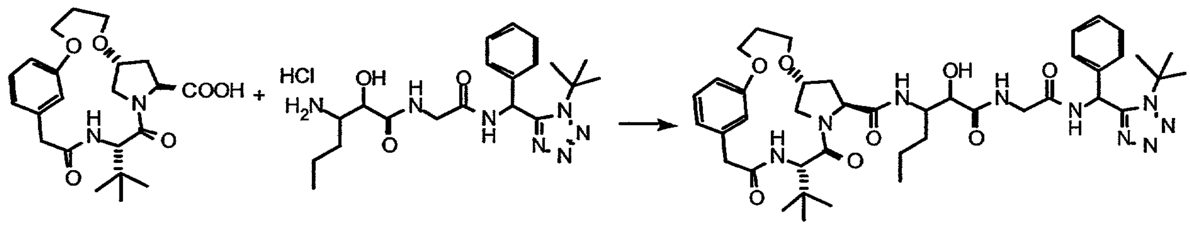
The acid **20e** was synthesized as follows:

An aqueous sodium hydroxide solution (0.45g in 30 ml H_2O) was added to a 0°C solution of compound **20e** in THF (30 ml) and methanol

(30 ml). This mixture was stirred in an ice bath and warmed to room temperature along with it in 4h. The progress of the reaction was monitored by TLC. After the volatiles were removed in vacuo, EtOAc (150 ml) and water (30 ml) were added and the two layers separated. The aqueous solution was saturated with solid sodium chloride. After separation of the layers, the aqueous layer was extracted with EtOAc (2 x 150 ml). Organic solutions were combined, dried with magnesium sulfate, filtered and concentrated in vacuo to afford compound **20e**.

Example 21

Step A

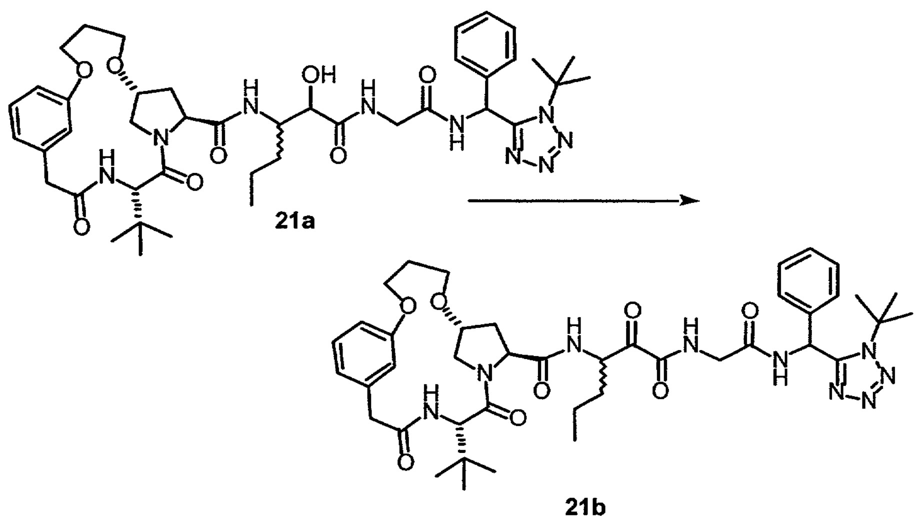


21a

A solution of the product of Example 19 (62 mg, 0.148 mmols) in dry DMF (2.5 mL) was treated with HOOBt (37 mg, 0.22 mmols) and NMM (58 mg, 0.592 mmols,) The reaction mixture was cooled to 0° C and treated with EDCI (63 mg, 0.33 mmols, 1.5 equiv) and stirred for 20 min. The reaction mixture was treated with the product of Example [11Q2] step B (74 mg, 0.0.16 mmols,) and stirred at rt for 48 h. The reaction mixture was concentrated in vacuo and diluted with H_2O (30 mL). The aqueous layer was extracted with CH_2Cl_2 (3x50 mL) and EtOAc(3x50 mL). The combined organic layers were extracted with aq. HCl (2M), aq. NaOH (2M), dried (Na_2SO_4) filtered concentrated in vacuo to obtain a colorless

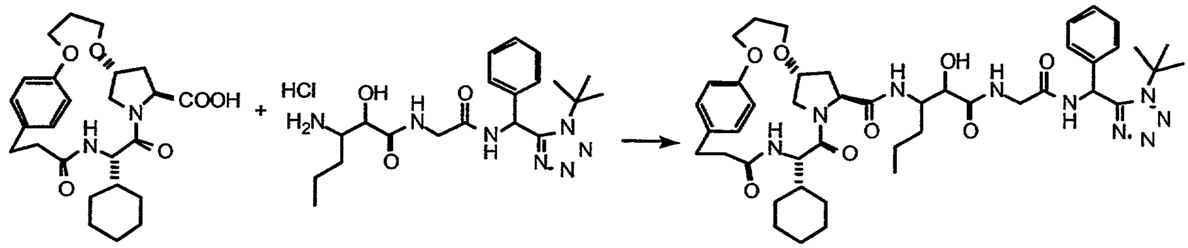
solid (120 mg) which was used for oxidation. MS: (Electron spray, *m/z rel int*): **818** [(M+1^{+}, 100].

Step B



A solution of the product of the preceding step (130 mg, 0.16 mmols) in CH_2Cl_2 (2.0 mL) was treated with Dess-Martin reagent (mg, 0.32 mmol, 2.0 equiv.). The reaction mixture was stirred at room temperature for 2 h and the mixture was concentrated in vacuo. The residue was purified by preparative TLC (SiO_2, CH_3OH/CH_2Cl_2 1:49) to yield oxidized product (55 mg, 42%) as a colorless solid. MS: (Electron spray, *m/z rel int*): 816 [(M +1)$^{+}$, 100].

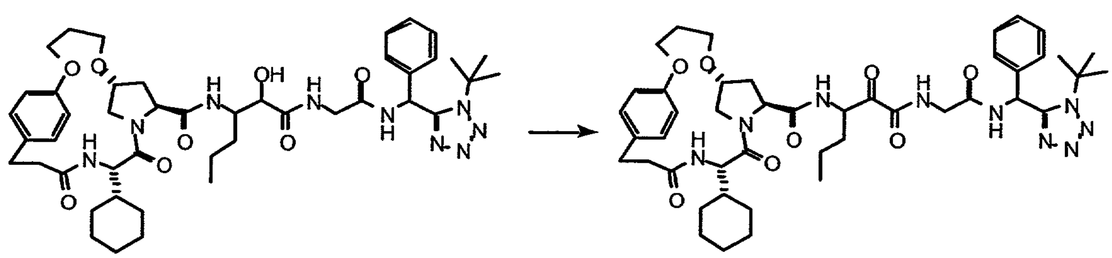
Example 22
Step A



21a

Following the procedure of Example 21 Step A, the product of Example 20, labeled **20e** is reacted with the product of Example 13 Step B to afford the corresponding compound as a colorless solid product which was used for oxidation; MS: [electron spray, *m/z(rel int*)] 858 [(M+1)+, 100], 604 (10), 446 (10).

Example 23
Step B



Following the procedure of Example 21 Step B., the product of the preceding Step was converted to the corresponding product as a colorless solid. MS: [electron spray, m/z(rel int)] 856 [(M+1)+,100].

Assay for HCV Protease Inhibitory Activity:

Spectrophotometric Assay: Spectrophotometric assay for the HCV serine protease was performed on the inventive compounds by following the procedure described by R. Zhang *et al*, *Analytical Biochemistry*, 270 (1999) 268-275, the disclosure of which is incorporated herein by reference. The assay based on the proteolysis of chromogenic ester substrates is suitable for the continuous monitoring of HCV NS3 protease activity. The substrates were derived from the P side of the NS5A-NS5B junction sequence (Ac-DTEDVVX(Nva), where X = A or P) whose C-terminal carboxyl groups were esterified with one of four different chromophoric alcohols (3- or 4-nitrophenol, 7-hydroxy-4-methyl-coumarin, or 4-phenylazophenol). Presented below are the synthesis, characterization and application of these novel spectrophotometric ester substrates to high throughput screening and detailed kinetic evaluation of HCV NS3 protease inhibitors.

Materials and Methods:

Materials: Chemical reagents for assay related buffers were obtained from Sigma Chemical Company (St. Louis, Missouri). Reagents for peptide synthesis were from Aldrich Chemicals, Novabiochem (San Diego, California), Applied Biosystems (Foster City, California) and Perseptive Biosystems (Framingham, Massachusetts). Peptides were synthesized manually or on an automated ABI model 431A synthesizer (from Applied Biosystems). UV/VIS Spectrometer model LAMBDA 12 was from Perkin Elmer (Norwalk, Connecticut) and 96-well UV plates were obtained from Corning (Corning, New York). The prewarming block was from USA Scientific (Ocala, Florida) and the 96-well plate vortexer was from Labline Instruments (Melrose Park, Illinois). A Spectramax Plus microtiter plate reader with monochrometer was obtained from Molecular Devices (Sunnyvale, California).

Enzyme Preparation: Recombinant heterodimeric HCV NS3/NS4A protease (strain 1a) was prepared by using the procedures published previously (D. L. Sali *et al*, *Biochemistry*, 37 (1998) 3392-3401). Protein concentrations were

determined by the Biorad dye method using recombinant HCV protease standards previously quantified by amino acid analysis. Prior to assay initiation, the enzyme storage buffer (50 mM sodium phosphate pH 8.0, 300 mM NaCl, 10% glycerol, 0.05% lauryl maltoside and 10 mM DTT) was exchanged for the assay buffer (25 mM MOPS pH 6.5, 300 mM NaCl, 10% glycerol, 0.05% lauryl maltoside, 5 µM EDTA and 5 µM DTT) utilizing a Biorad Bio-Spin P-6 prepacked column.

Substrate Synthesis and Purification: The synthesis of the substrates was done as reported by R. Zhang *et al*, (*ibid.*) and was initiated by anchoring Fmoc-Nva-OH to 2-chlorotrityl chloride resin using a standard protocol (K. Barlos *et al*, *Int. J. Pept. Protein Res.*, 37 (1991), 513-520). The peptides were subsequently assembled, using Fmoc chemistry, either manually or on an automatic ABI model 431 peptide synthesizer. The N-acetylated and fully protected peptide fragments were cleaved from the resin either by 10% acetic acid (HOAc) and 10% trifluoroethanol (TFE) in dichloromethane (DCM) for 30 min, or by 2% trifluoroacetic acid (TFA) in DCM for 10 min. The combined filtrate and DCM wash was evaporated azeotropically (or repeatedly extracted by aqueous Na_2CO_3 solution) to remove the acid used in cleavage. The DCM phase was dried over Na_2SO_4 and evaporated.

The ester substrates were assembled using standard acid-alcohol coupling procedures (K. Holmber *et al*, *Acta Chem. Scand.*, B33 (1979) 410-412). Peptide fragments were dissolved in anhydrous pyridine (30-60 mg/ml) to which 10 molar equivalents of chromophore and a catalytic amount (0.1 eq.) of para-toluenesulfonic acid (pTSA) were added. Dicyclohexylcarbodiimide (DCC, 3 eq.) was added to initiate the coupling reactions. Product formation was monitored by HPLC and found to be complete following 12-72 hour reaction at room temperature. Pyridine solvent was evaporated under vacuum and further removed by azeotropic evaporation with toluene. The peptide ester was deprotected with 95% TFA in DCM for two hours and extracted three times with anhydrous ethyl ether to remove excess chromophore. The deprotected substrate was purified by reversed phase HPLC on a C3 or C8 column with a 30% to 60% acetonitrile gradient (using six column volumes). The overall yield following HPLC purification

was approximately 20-30%. The molecular mass was confirmed by electrospray ionization mass spectroscopy. The substrates were stored in dry powder form under desiccation.

<u>Spectra of Substrates and Products:</u> Spectra of substrates and the corresponding chromophore products were obtained in the pH 6.5 assay buffer. Extinction coefficients were determined at the optimal off-peak wavelength in 1-cm cuvettes (340 nm for 3-Np and HMC, 370 nm for PAP and 400 nm for 4-Np) using multiple dilutions. The optimal off-peak wavelength was defined as that wavelength yielding the maximum fractional difference in absorbance between substrate and product (product OD - substrate OD)/substrate OD).

<u>Protease Assay:</u> HCV protease assays were performed at 30°C using a 200 µl reaction mix in a 96-well microtiter plate. Assay buffer conditions (25 mM MOPS pH 6.5, 300 mM NaCl, 10% glycerol, 0.05% lauryl maltoside, 5 µM EDTA and 5 µM DTT) were optimized for the NS3/NS4A heterodimer (D. L. Sali *et al, ibid.*)). Typically, 150 µl mixtures of buffer, substrate and inhibitor were placed in wells (final concentration of DMSO 4 % v/v) and allowed to preincubate at 30 °C for approximately 3 minutes. Fifty µls of prewarmed protease (12 nM, 30°C) in assay buffer, was then used to initiate the reaction (final volume 200 µl).The plates were monitored over the length of the assay (60 minutes) for change in absorbance at the appropriate wavelength (340 nm for 3-Np and HMC, 370 nm for PAP, and 400 nm for 4-Np) using a Spectromax Plus microtiter plate reader equipped with a monochrometer (acceptable results can be obtained with plate readers that utilize cutoff filters). Proteolytic cleavage of the ester linkage between the Nva and the chromophore was monitored at the appropriate wavelength against a no enzyme blank as a control for non-enzymatic hydrolysis. The evaluation of substrate kinetic parameters was performed over a 30-fold substrate concentration range (~6-200 µM). Initial velocities were determined using linear regression and kinetic constants were obtained by fitting the data to the Michaelis-Menten equation using non-linear regression analysis (Mac Curve Fit 1.1, K. Raner). Turnover numbers (k_{cat}) were calculated assuming the enzyme was fully active.

Evaluation of Inhibitors and Inactivators: The inhibition constants (K_i) for the competitive inhibitors of Table A were determined experimentally at fixed concentrations of enzyme and substrate by plotting v_o/v_i vs. inhibitor concentration ($[I]_o$) according to the rearranged Michaelis-Menten equation for competitive inhibition kinetics: $v_o/v_i = 1 + [I]_o/(K_i (1 + [S]_o/K_m))$, where v_o is the uninhibited initial velocity, v_i is the initial velocity in the presence of inhibitor at any given inhibitor concentration ($[I]_o$) and $[S]_o$ is the substrate concentration used. The resulting data were fitted using linear regression and the resulting slope, $1/(K_i(1+[S]_o/K_m)$, was used to calculate the K_i value.

The obtained K_i values for various compounds of the present invention are given in the afore-mentioned **Table** wherein the compounds have been arranged in the order of ranges of K_i values. From these test results, it would be apparent to the skilled artisan that the compounds of the invention have excellent utility as NS3-serine protease inhibitors.

While the present invention has been described with in conjunction with the specific embodiments set forth above, many alternatives, modifications and other variations thereof will be apparent to those of ordinary skill in the art. All such alternatives, modifications and variations are intended to fall within the spirit and scope of the present invention.

Table A - Serine Protease Inhibitory Activity

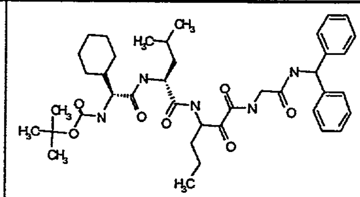
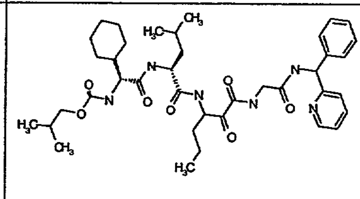
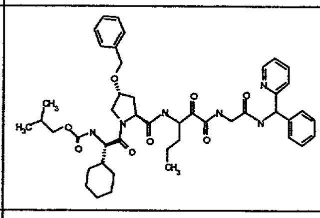
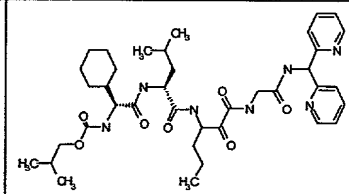
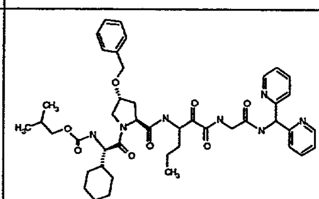
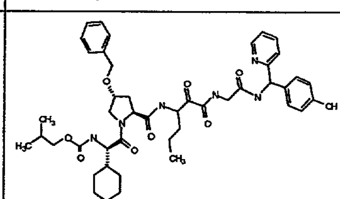
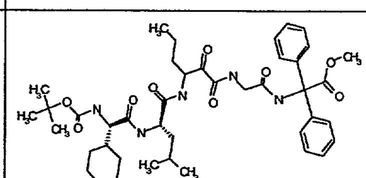
			HCV Assay Range
Ex.	structure	MolWt.	Ki* (nM)
I		719.93	d
II		720.92	c
III		811.00	b
IV		721.90	c
V		811.99	b
VI		827.00	c
VII		777.97	c

Table A - Serine Protease Inhibitory Activity

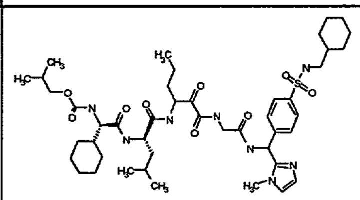
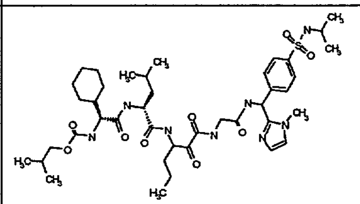
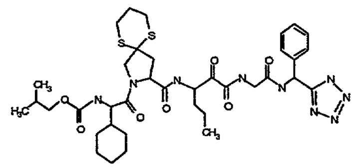
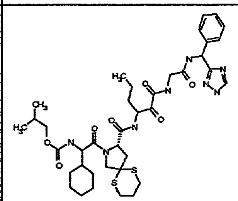
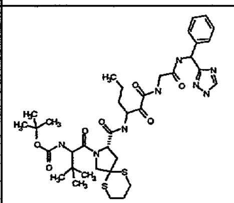
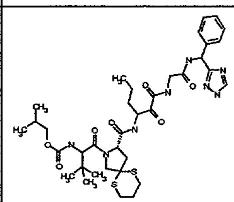
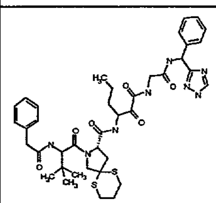
			HCV Assay Range
Ex.	structure	MolWt.	Ki* (nM)
VIII		899.17	d
IX		845.08	c
X		800.02	a
XI		799.03	b
XII		772.99	a
XIII		772.99	b
XIV		791.01	b

Table A - Serine Protease Inhibitory Activity

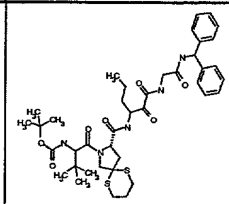
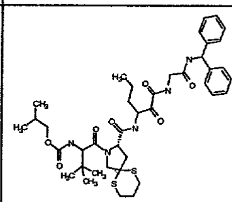
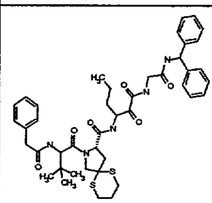
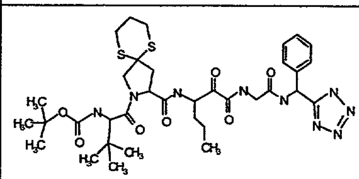
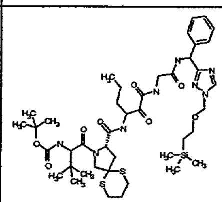
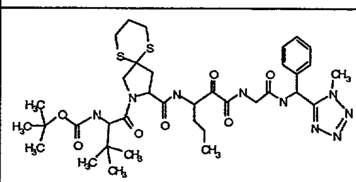
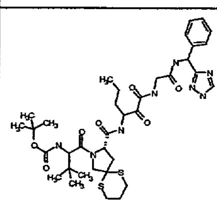
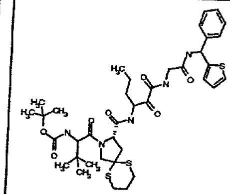
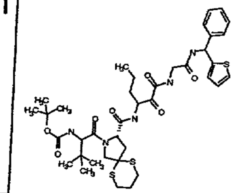
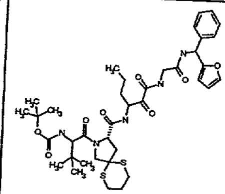
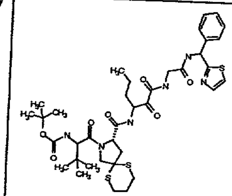
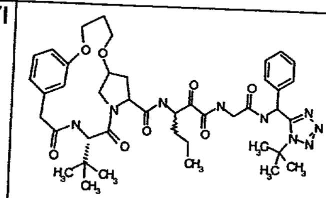
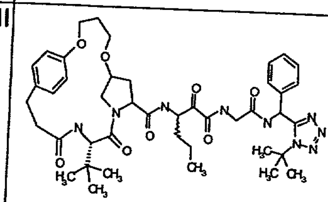
			HCV Assay Range
Ex.	structure	MolWt.	Ki* (nM)
XV		782.04	b
XVI		782.04	b
XVII		800.06	b
XVIII		773.98	b
XIX		903.26	c
XX		788.01	b
XXI		772.99	a

Table A - Serine Protease Inhibitory Activity

Ex.	structure	MolWt.	HCV Assay Range Ki* (nM)
XXII		788.07	b
XXIII		788.07	c
XXIV		772.00	b
XXV		789.05	b
XXVI		815.98	b
XXVII		830.01	c
	HCV Assay Ki* range: Category a=10-99nM; b=100-999nM; c=1000-9999nM; d=10,000-50,000nM		